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THE EFFECT OF ETHANOL ON THE TOXICITY OF HEXOBARBITAL, THIOPENTAL, MORPHINE, ATROPINE AND SCOPOLAMINE

AN EXPERIMENTAL STUDY ON MICE

BY

RISTO EEROLA

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MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

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Tampere, April 1961.

Risto Eerola

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I. INTRODUCTION

Wine was man's earliest intoxicant and anodyne; in all ages it was known that the narcotic effect of drunkenness enabled surgical operations to be performed without pain. In the days of Homer and Pliny the sedative action of some herbs, such as nepenthes and helenium, was well known, and they were very often taken in wine. In the *Odyssey* Homer describes the medical knowledge of Helen of Troy as follows: »Straightway she cast into the wine of which they were drinking a drug to quiet all pain and strife, and to bring forgetfulness of every ill.» After the discovery of opium and mandragora these drugs were used in combination with wine to relieve pain (Robinson 1947). In the first century A.D. Dioscorides mentioned the combined action of opium and mandragora; and the synergistic action of opium and hyoscyamus niger was described in the medieval drug book *Circa Instans* (Hendrych 1936). These are the first mentions in the literature of the combined action of two drugs.

The antagonistic action of two agents, atropine and physostigmine, was discussed by Fraser (1872 a, b). According to him antagonism was a well known phenomenon as early as 1570, when Pena and De Lobel told that »Italian vagabonds, who gained much notoriety by employing the root of the belladonna plant to quench thirst were in the habit of administering opiates to remedy the evil effects that occasionally were thereby produced».

The combined action of two drugs administered together may cause a great variety of effects in the organism. To describe the nature of this combined action certain terms are used in the literature, but there are a great many inconsistencies in the use of this terminology by different investigators and writers. Uniformity of terms has not been attained.

The following definition of the combined action is given by Goodman and Gilman (1941): »When the combined action of two drugs administered at the same time is equal to the algebraic sum of their individual effects the response is known as *summation*. Summation can be negative or positive. Negative summation is also termed *antagonism*. — Positive summation is known as *synergism*. — There are instances in which the combined action of two drugs is greater than that which can be anticipated from the sum of their individual actions. This is known as *potentiation*.«

According to Sollman (1957) the terminology is defined as follows: »When several drugs are administered together each may act independently as if it were present alone. The result would then be a *simple algebraic summation* of the effects, either synergistic or antagonistic. In many cases, however, the combined action is greater or smaller than would be calculated (*potentiated and deficient summation*).«

Møller (1958) defines the terms as follows: »Wenn zwei *Stoffe mit Wirkung auf die gleichen Funktionen* gleichzeitig verabreicht werden, können sie entweder eine gesteigerte (*synergistische*) oder eine verminderte (*antagonistische*) Wirkung entfalten. Ein *Synergismus* wird erzielt wenn die beiden Stoffe dieselben Funktionen oder dasselbe Organ auf gleiche oder praktisch gleiche Weise beeinflussen, so dass die Wirkungen summiert werden. Wenn z.B. mit der halben narkotischen Dosis eines Stoffes und der halben narkotischen Dosis eines anderen Stoffes eine volle Narkose erzielt, so spricht man von einer *additiven Wirkung* (algebraische Summation). Wenn man aber eine Narkose z.B. mit einem Drittel der narkotischen Dosis der beiden Stoffe ($1/3 + 1/3 = 2/3$ d.h. < 1), so spricht man von einer *potenzierten Wirkung* (potenzierte Summation). *Antagonismus:* Darunter versteht man die Tatsache dass zwei Stoffe mit Wirkung auf die gleichen Funktionen sich gegenseitig hemmen bzw. gegenseitig in ihrer Wirkung aufheben.«

In conformity with Fraser (1872 b) and Loewe (1928) Gaddum (1959) illustrates the combined action of two drugs with diagrams. His terms are generally used in the English literature. Fig. 1 is a diagrammatic presentation of the synergistic effect of two drugs. The axes denote the concentrations of the drugs: OA stands for the drug A, OB for the drug B. If the point representing their

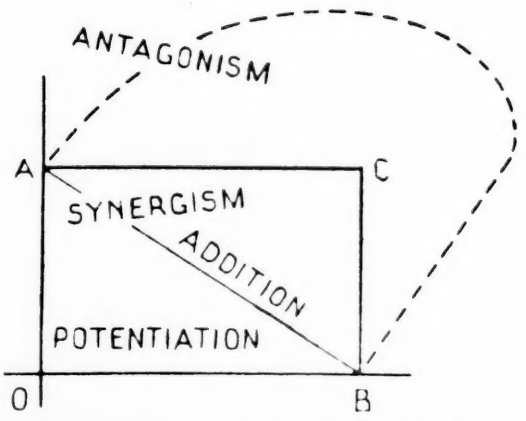


Fig. 1. — Diagram showing the terms used to describe the combined effects of two drugs. The dose of one drug is plotted on the horizontal axis and OB represents the dose which is just enough to have some definite effect, such as anaesthesia or death. OA represents the corresponding dose of the other drug. (Gaddum 1959).

combined action falls within the quadrangle OACB, the action of the drugs is *synergistic*. If the point falls on the straight line AB, the action is called *additive* (summation). If the point denoting the combined action falls within the triangle OAB, it shows that the combined effect of the drugs is greater than the simple addition of their individual effects. This is known as *potentiation*. If the point falls outside the lines AC and CB, there exists *antagonistic* action.

To summarize: The definitions given by Goodman and Gilman and by Sollman agree in principle. Møller formulates the terms more exactly and holds that we can speak of synergism only when both the drugs have a similar effect on the same actions or on the same organ. He also introduces the term addition besides summation. Gaddum gives a diagrammatic representation of all the above terms.

There is even discrepancy between definitions in different medical dictionaries. Stedtman (1957) defines synergism simply as »co-operation in action» between two drugs, and potentiation as »enhancing the potency of an agent». Dorland (1959), on the other hand, gives the following definition of synergism: »the joint action of agents, so that their combined effect is greater than the algebraic sum of their individual effects.» According to this dictionary potentiation is synonymous with synergism.

The investigators who have dealt with the combined action of drugs seem to have come to conflicting conclusions, some of which may be due to the heterogeneity of the terminology.

Since 1909 Bürgi and his school have published several papers on the combined effect of two drugs (1909, 1910, 1911). Bürgi has formulated his opinion about the combined action of two drugs in the law known by his name: »Eine Mehrheit von im grossen und ganzen gleichartig wirkenden Arzneien löst im tierischen Organismus nur dann einen ungewöhnlich höhen über dem Additionsergebnis der Einzeleffecte liegenden Gesamteffect aus, wenn die einzelnen Glieder der Medikamentmischung unter verschiedene, pharmakologische Angriffspunkte zeigen bei gleichzeitiger Einfuhr in den Tierleib eine glatte Addition ihrer Einzelwirkungen» (1912). In his paper of 1916 he again expresses his explicit opinion that only drugs with a similar mode of action can have an additive effect when administered together. The combined action can be greater than the sum of the individual actions only on condition that the mode of action of the drugs is dissimilar. As an example he uses two groups of narcotics with dissimilar modes of action: aliphatic hydrocarbons and phenantrene derivatives. Besides, he emphasizes that the variations must be sufficiently great to be considered. An increase of 10—20 per cent in the effect is too small to be regarded as potentiation, because such an increase may be caused by variations in the experimental conditions. Bürgi considered it exceedingly difficult to prove potentiation. He insisted on carrying out a great number of experiments; only strong deviations can be counted and there are marked differences between man and animals.

Bürgi's law was confirmed by the results of several investigators (Hauckold 1910, Bojarski 1916, Giesel 1916, Lewin 1916). On the other hand, there were others (Breslauer and Woker 1912, Issekutz 1915) who seemed to prove potentiation in their studies of narcotics with similar modes of action. Mayer and Gottlieb (1933) agreed with Bürgi in their opinion about the combined action of drugs.

Clark (Heubner and Schüller 1937) discusses widely the problem of the combined action of drugs. According to him the action of every pair of drugs is more or less synergistic or antagonistic. Potentiation is a much weaker action than antagonism. He isolates three different types of antagonism: 1. Chemical antagonism. The

drugs react *in vitro* and the resultant has a weaker action than the original drugs. 2. Physiological antagonism. Each drug acts in the cells independently in different directions. 3. Specific antagonism. One drug prevents the action of the other in the cells, although there is no reaction between the drugs *in vitro*. After Clark the majority of the antagonism cases could be fitted into these groups. He classifies the synergism of drugs similarly.

Everything mentioned above serves to indicate that in pharmacology the investigation of the combined action of drugs, with its various problems, is of great importance. These problems are made even more difficult if the drugs used include some whose doses are uncertain or unknown; when the doses vary the combined action may change from antagonism to synergism, or from the additive type of synergism to potentiation.

The combined action of ethanol and other drugs is most important for various reasons. A person under the influence of ethanol may simultaneously use other drugs such as hypnotics; the physician may also be compelled to give such a person sedatives or premedicants before an operation due to an acute injury. Clinical experience of the combined effects of ethanol and barbiturates or ethanol and morphine has clearly proved these problems to be most important.

The first to pay attention to the combined action of ethanol and barbiturates in man was Fühner (1930). He described one case where a person had taken a glass of strong wine against a cold and at the same time a tablet of Quinisal (chinium bisalicylosalicylicum) and one tablet of propallylonal (Noctal)[1]. He fell into a deep coma from which he recovered with careful treatment.

Human experiments were carried out by Elbel (1938) and Peter (1939). Elbel's subjects were given 3/4 l. of 8.8 per cent wine during 30 minutes and 0.5 gm. of barbital (Veronal) two hours afterwards. Their physical capacities were then tested using a ring test. Small rings were put on a stick and the speed and the number of mistakes were counted. No marked effect was discovered but the results gave some evidence of the increase of the intoxication after administration of barbital. Peter's subjects were given ethanol

[1] As there are great varieties in the nomenclature of the barbiturates, the pharmaceutical and chemical names of the barbiturates appearing in the text are listed in Table 1.

TABLE 1

Trade name	Pharmaceutical name	Chemical name
Amytal	Amobarbital (U.S.P. XVI)	5-Ethyl-5-isoamylbarbituric acid
Evipal sodium	Hexobarbital (N.F. XI)	Sodium 5-(1-cyclohexen-1-yl)-1,5-dimethylbarbiturate
Gardenal Luminal	Phenobarbital (U.S.P. XVI)	5-Ethyl-5-phenyl barbituric acid
Kemithal	Thialbarbitone (————)	5-Allyl-5-(cyclohexen-2-yl) 2-thiobarbituric acid
Nembutal	Pentobarbital (N.N.D. 1960)	5-Ethyl-5-(1-methylbutyl) barbituric acid
Neonal	Butethal (N.F. X)	5-Ethyl-5-n- butyl barbituric acid
Noctal	Propallylonal (————)	5-(2-bromallyl)-5-isopropylbarbituric acid
Pentothal sodium	Thiopental sodium (U.S.P. XVI)	Sodium 5-ethyl-5-(1-methylbutyl)-2-barbiturate
Pernocton	Butallylonal (N.F. X)	β-Bromallyl-sec-butylbarbituric acid
Phanodorm	Cyclobarbital (N.F. X)	5-(1-cyclohexenyl)-5-ethylbarbituric acid
Seconal	Secobarbital (U.S.P. XVI)	5-Allyl-5-(1-methylbutyl) barbituric acid
Veronal	Barbital (U.S.P. XIV)	Diethylbarbituric acid

and phenobarbital or ethanol and Cibalgine (pyramidon 0.22 gm. + diallylbarbituric acid 0.03 gm.). In one of the tests the person received 0.3 gm. of phenobarbital and 1½ hours later 1 gm. of ethanol per kg. of body weight; on another occasion he was given 0.75 gm. of Cibalgine and 1 hour afterwards 1 gm. of ethanol per kg. The combined phenobarbital — ethanol experiment revealed a considerably greater decrease in the physical abilities than after the administration of ethanol or phenobarbital alone. The combined Cibalgine — ethanol test showed a sharp decrease in physical capacities after the administration of ethanol. The subject developed a deep intoxication, which deepened into a narcosis from which he woke after several hours.

As a cure for psychoneuroses Miller (1952) recommended intravenous doses of 20—25 ml. of 10—15 per cent of ethanol together with 120—230 mg. of amobarbital (Amytal). Bartley (1953) also

suggested intravenous administration of thiopental to calm down restless intoxicated patients. On the other hand, Nobes (1953), Pullar-Strecker (1953), and Feldman and Zucker (1953) gave serious warnings against the simultaneous use of ethanol and barbiturates. The Russian Raschek (1945), however, made good use of the combined administration of ethanol and hexobarbital in intravenous anaesthesia.

Fatalities following the combined use of ethanol and barbiturates have been reported by Jetter and McLean (1943), Fischer *et al.* (1948) and Burrows (1953). Jetter and McLean reported three cases, Fischer *et al.* four, Burrows two; in all of these cases the combined action of ethanol and barbiturates was found to be the obvious cause of death.

Møller (1952) drew attention to those cases in which the patient who was under the influence of ethanol suddenly and unexpectedly died after receiving a therapeutic dose of morphine or morphine and scopolamine. He reported 12 cases in which the causal connection of the combined effect of ethanol and morphine with death was fairly certain. Naalsund (1955) also published a report on two cases in which death was indisputably caused by the combined action of ethanol and morphine.

Wagner and Wagner (1958) also paid attention to those fatalities which occurred after the administration of barbiturates or morphine or morphine-like drugs to persons under the influence of ethanol. They reported a case where a male nurse who was a Polamidon addict (dl-2-dimethyl amino-4,4 diphenyl-heptanon-(5) hydrochloride) suddenly died after an intravenous injection of 33 mg. of Polamidon; he had been drinking heavily for several days.

II. THE SUBJECT OF INVESTIGATION

The above facts serve to prove that the combined effects of ethanol and barbiturates, and those of ethanol and morphine, are noteworthy factors in man. Whether the combined action of these drugs in man is additive or potentiative is not easily solved clinically. Animal experiments elucidate some sides of the problem.

Modern anaesthetists make very frequent use of ultra short acting barbiturates, which explains the choice of hexobarbital and thiopental as subjects of this study. In addition to morphine I have chosen atropine and scopolamine, whose combined action with ethanol must also be studied, because all of them may be administered to patients under the influence of ethanol, either as premedication or combined with morphine as a sedative.

The earlier investigations of the combined action of ethanol and barbiturates have afforded inconsistent results; morphine has been the subject of only two studies; and atropine and scopolamine have remained outside the scope of these investigations. In consequence, the following experiments have been considered warranted.

The aim of this work is to try to answer the following questions: Are the combined effects of the following drugs antagonistic or synergistic; if synergistic, is the synergism of the additive or potentiative type?

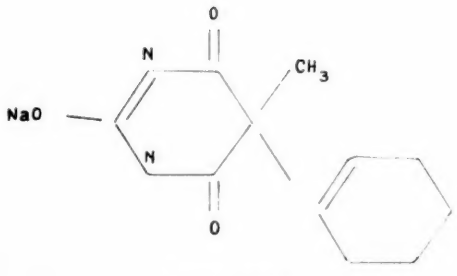
1. Ethanol and hexobarbital
2. Ethanol and thiopental
3. Ethanol and morphine
4. Ethanol and atropine
5. Ethanol and scopolamine

The chemical structures of the drugs used are presented in Table 2.

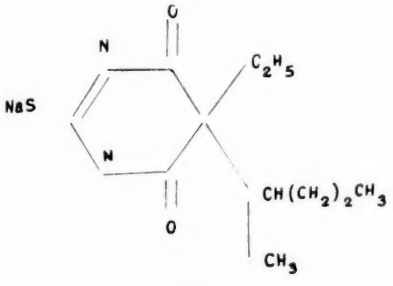
TABLE 2

$CH_3 \cdot CH_2OH$

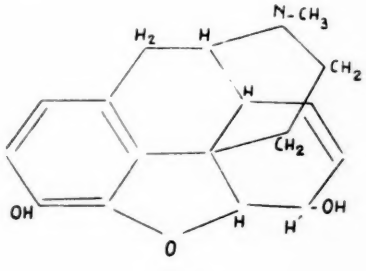
Ethanol



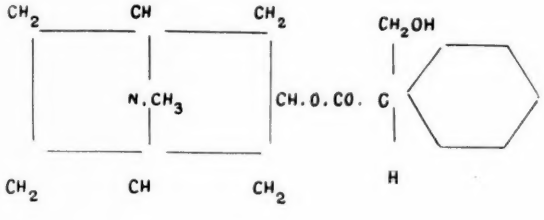
Hexobarbital



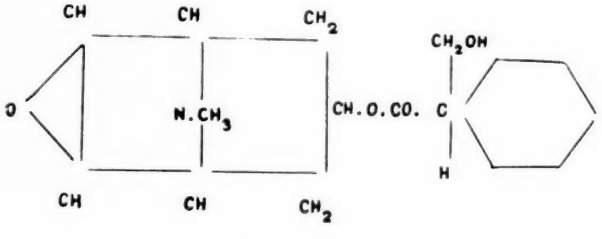
Thiopental



Morphine



Atropine



Scopolamine

III. REVIEW OF THE LITERATURE

A. PREVIOUS METHODS

In toxicity experiments the mouse is a common test animal, because it is cheap and of suitable size in comparison with many other laboratory animals (Weese 1928, Evertsbusch and Geiling 1956). According to Weese there is no fundamental difference between mice and other test animals for ethanol experiments.

Nearly all the mouse experiments have been made on animals of one *breed*, the importance of which is particularly emphasized by Schmitz (1926) and by Latven and Molitor (1939). For example in Schmitz's experiments the lethal dose of morphine (1.2 mg. per gm.) was twice the dose reported by other investigators.

Differences may also be due to diversities in *food*. Robinson (1945) fed the mice on two different diets and found the difference to be of some importance. During the experiments the mice were not generally fed at all. Some investigators, however, like Gosselin *et al.* (1955) gave water *ad libitum*, but then the mice were starved for 18—24 hours before the experiments.

In his experiments Schmitz endeavoured to keep the *outer conditions* as constant as possible. Thus he made his experiments in January, February and March in order to eliminate the effect of changes of the seasons. Also Marshall and Owens (1955), when determining the metabolism of ethanol in mice, kept the room temperature constant (28°C). They discovered that at a temperature of 15—18°C there were fluctuations in the concentrations of ethanol, so that blood samples from the heart revealed higher concentrations than those taken from the tail. At the temperature of 28°C, on the other hand, the respective samples gave equal concentrations.

All the investigators have not paid attention to the *sex* of the mice. Glassman and Seifert (1955), Gruber (1955), Wagner and

Wagner (1958), for example, experimented on both female and male mice, using both sexes in equal numbers. Most investigators, however, have used either female or male mice exclusively. Nelson *et al.* (1957), Aston and Cullumbine (1959), for example, used only females; Fearn and Hodges (1953), Gosselin *et al.* (1955), Marshall and Owens (1955), Evertsbusch and Geiling (1956), and Aull *et al.* (1956) experimented only on male mice. Remmer (1958) paid attention to the much greater tolerance of male rats for hexobarbital. In Gruber's experiments with ethanol and barbiturates the sleeping time of the males was a little longer than that of the females, but there was no difference in the death frequencies. In the rat experiments carried out by Mensch and Jongh (1959) there was no significant difference between the reactions of the sexes.

In various series of experiments the *weight* of the mice has ranged from 13 to 35 gm. Caesar (1912) discovered that mice weighing 15—25 gm. showed constant sensitiveness to morphine. The mice were weighed to an accuracy of 1 gm. immediately before the experiment; but Schmitz (1926) and Latven and Molitor (1939), for example, weighed their mice to an accuracy of 0.5 gm. The weights of the latter varied between 15 and 22.5 gm.

The *method of administration* also has its effect upon the result. In experiments of this kind ethanol has been given to mice as follows: intravenously via the tail vein; subcutaneously in the neck region, or in the abdominal region; intraperitoneally; or orally using a cannula direct into the stomach. In each series of experiments referred to the same method of administration was used throughout in order to preserve the comparability of the results.

Latven and Molitor made a comparative [illegible] of the toxicity of ethanol on mice when administered intravenously, subcutaneously and orally. The intravenous injection was given via the tail vein and the subcutaneous one in the abdominal region; the oral dose was introduced using an injection needle with its point cut and shielded with a piece of cement. The ethanol was used in 70 and 95 per cent solutions, and the LD_0, LD_{50} and LD_{100} were determined for each series of mice. They discovered that toxicity was highest for the intravenous administration and smallest for the oral method. Table 3 illustrates their results.

TABLE 3

	Subcutaneous			Peroral			Intravenous		
	LD_0	LD_{50}	LD_{100}	LD_0	LD_{50}	LD_{100}	LD_0	LD_{50}	LD_{100}
Ethanol 70 %..	11.0	14.5	18.0	13.0	14.5	17.0	3.0	4.0	5.0
Ethanol 95 %..	9.0	10.5	12.0	9.0	12.0	14.0	2.0	2.5	3.0

(Latven and Molitor 1939)

Marshall and Owens (1955) and Aston and Cullumbine (1959) also stated that the intravenous administration produces greater toxicity than other methods of administration.

Unlike the investigators mentioned above, Weese (1928) and Sandberg (1951) used ethanol inhalations in their experiments.

Matsuda and Yamaguti (1940) compared the toxic effect of morphine in mice by intravenous, intraperitoneal and subcutaneous ways of administering. Their results are shown in Figs. 2 and 3. Morphine was found to be most toxic when given intravenously and the toxicity varied in narrower limits. The intraperitoneal injection produced greater toxicity than the subcutaneous one, but the toxicity curve was similar in shape.

When investigating the metabolism of radioactive atropine Gosselin *et al.* (1955) found no difference between the intravenous method and the intraperitoneal one as regards the excretion of atropine during the first two hours after administration.

In his experiments on mice Raventós (1938) paid attention to the fact that in giving barbiturates intravenously the injection rate was of considerable importance. After an injection of 1 minute's duration the LD_{50} was 150—180 mg per kg., whereas after a fast injection it was 70 mg. per kg. The same fact was pointed out by Robinson (1945). He determined the relation between the anaesthetic dose and the lethal dose by giving mice intravenous injections of barbiturates. (Fig. 4).

For oral administration the Behrens method has been used by most investigators. Behrens (1924) found the mouse an advantageous test animal for oral administration. After 1 hour its stomach was already empty, and it never vomited. Its stomach was relatively large, and it easily held 1.5 ml. of liquid. Behrens used a nickel cannula, 7 cm. in length and 0.75 mm. in diameter. Its lower end was soldered in and rounded like a ball. Immediately

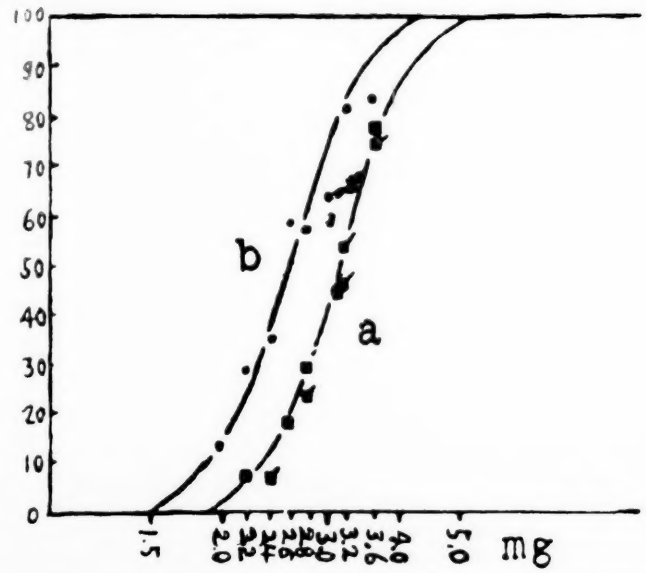


Fig. 2. The mortality curve for mice after the intravenous injection of morphine. Abscissa: The percentage of dead mice. Ordinate: Log of the morphine dose per 10 gm. of body weight. a: The number of mice that died due to respiratory paralysis after the injection; morphine addicts and non-addicts. b: The number of mice that died after 1 hour due to convulsions. (Matsuda and Yamaguti 1940).

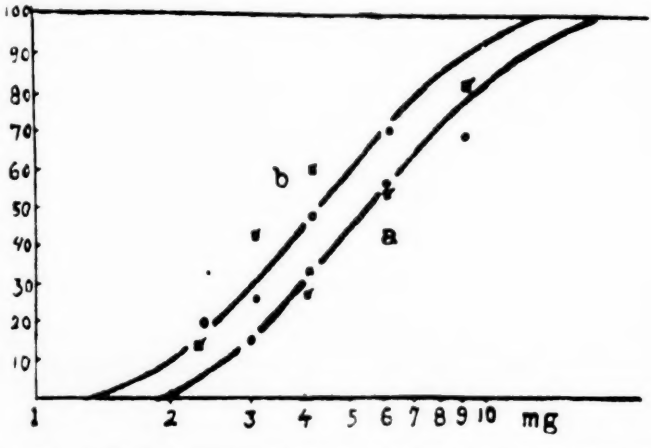


Fig. 3. — The mortality curve after (a) subcutaneous and (b) intraperitoneal morphine injections in morphine addicted mice. Abscissa: Percentage of the dead mice. Ordinate: Log of the morphine dose per 1 gm. of body weight. (Matsuda and Yamaguti 1940)

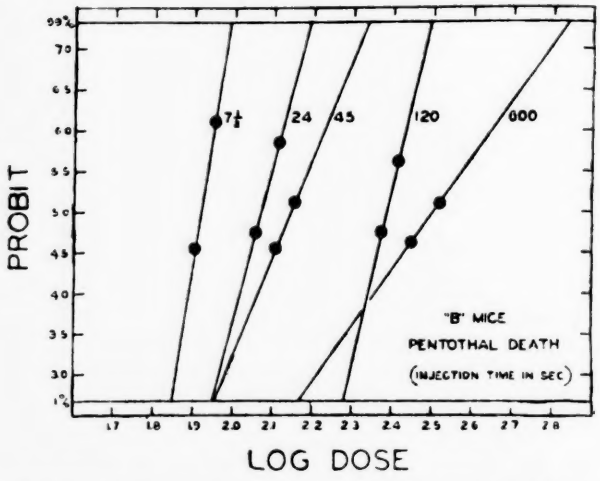


Fig. 4. — Relation between slope of dosage-effect curve and injection time. Abscissa illustrates log probit scale of mortality and ordinate log dose of thiopental. (Robinson 1945)

above the point a hole was made in the needle. 3.2 cm. from the point a mark was made to facilitate the insertion, and at 4.5 cm. another mark to indicate the arrival of the cannula in the stomach. Behrens fastened a syringe to the cannula beforehand to make the insertion easier and to simplify the injection. Less than 1 per cent of his experiments were failures.

As mentioned already, the *concentration* of the solution used also has a bearing on its toxicity (Latven and Molitor 1939, Hiestand *et al.* 1952, Aston and Cullumbine 1959).

In his toxicity experiments Gruber (1955) used an *observation period* of 12 hours, but a period of 24 hours is generally employed (Fearn and Hodges 1953, Hiestand *et al.* 1952, Graham 1960). The same period was used by Latven and Molitor to observe mortality, but in addition to this they employed one week to follow up the surviving test animals. The death cases which occurred within this period were called late deaths. After intravenous administration of thiopental Robinson's mice were observed for 20 minutes only (1945). Nearly all the animals were found dead after this period. The deaths which occurred a few hours or even days afterwards were caused by some complicating factor, like pneumonia. After an observation period of 18 hours the LD_{50} decreased only 7 per cent with an injection speed of 2 minutes,

and 20 per cent with an injection speed of 5—10 minutes. Ramsey and Haag (1946) observed their test animals for 48 hours.

The effects of ethanol, barbiturates, morphine, scopolamine and atropine upon mice have generally been checked in two ways: using either the sleeping time or death as the criterion. In a few cases the results were measured using changes in the pain threshold.

To measure the duration of the sleeping time a great variety of *methods* have been used. Latven and Molitor made their mice walk along a narrow lath, 1 cm. in breadth, to make changes in their behaviour more perceptible. Using this method they determined the hypnotic effect of ethanol, its minimum symptomatic dose. 15 minutes after intravenous administration Robinson pinched the tail of the mouse with his finger nail; if it took three successive steps after pinching it was not counted asleep. Sandberg (1951) determined the anaesthetic effect as follows: with 0.85 ml. of 99.5 per cent ethanol he moistened a piece of gauze tissue which was enclosed in a glass bottle holding 2.95 l. The ethanol was allowed to vapourize for 20 minutes. The room temperature during the experiment was 21—23°C. A mouse which had first been weighed (20—24 gm.) was then quickly put in. The bottle was then placed in a horizontal position and rolled, always by the same person, a distance of 1 metre in each direction at a constant speed. Three different degrees of anaesthesia were observed: a) the mouse rolled round once; b) it rolled round three times; c) it kept on rolling round passively. The time was counted from the moment the animal was put into the bottle till the onset of the different stages, and the times of induction were employed to measure the anaesthetic effect. The mice were divided into three groups, ten animals in each, and the experiments were arranged as follows: 1) ethanol inhalation; 2) subcutaneous injection of 0.100 mMol. of barbiturate per kg. immediately after the ethanol inhalation; 3) administration of 0.100 mMol. of barbiturate per kg. alone. All the statistical results were presented as logarithmic functions.

Mensch and Jongh (1958) used the following method: after an intravenous injection of 20 mg. of barbiturate per kg. the mouse was placed on its back in a warm jar. If it was able to get on its feet within 30 seconds, it was turned on its back again. This was repeated twice if needed. The absence of the righting reflex for 30 seconds was counted as a narcotic effect (all or none criterion).

The righting reflex was used as the criterion also by Smith and Loomis (1951), Fearn and Hodges (1953), Glassman and Seifert (1955) and Gruber (1955).

Wallgren (1960) investigated the effect of ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec. butyl and tert. butyl alcohols using the following method: After administration the rat was placed in an upright position on a rough-surfaced plate. The plate was tilted at different angles, and the degree of the angle at which the rat fell down was used as the measure of the intoxication caused by the alcohol.

Smith and Loomis (1951) studied thiopental, ethanol and their combined effect on mice, using as the criterion the pain threshold, as determined with the apparatus of Chen and Beckman. The animal was dropped into a boiling mixture of azeotropic ethyl-formate and acetone, the boiling point being 55°C. The animal was dropped in this receptacle 2 minutes after an intravenous injection of thiopental and ethanol. The time needed by the animal before it was able to lick its hind leg was taken as the reaction time.

Many investigators used death as the criterion for measuring toxicity. Generally the LD_{50} or the dose which produced death in 50 per cent of the animals was determined. Some earlier investigations were exceptions to this general practice for determining toxicity. For example, Schmitz's (1926) results were based on one series of experiments only, without any mathematical analysis. On the other hand, Latven and Molitor (1939) determined both the LD_0, LD_{50} and the LD_{100}. All the results were mathematically checked.

B. TOXICITY AND COMBINED ACTION OF THE DRUGS UNDER INVESTIGATION

1. ETHANOL

The literature contains several investigations which deal with the toxic effects of alcohols upon animals. As early as 1869 Richardson investigated the toxic effects of different alcohols upon pigeons, guinea-pigs and rabbits. He discovered that the toxicity of methyl, ethyl, propyl, butyl, and capryl alcohols was in direct proportion

to the number of their carbon atoms. Führer (1904) made an experimental study of the effects of different alcohols upon the development of sea urchin eggs and reported that propyl alcohol was 3 to 4 times as toxic as ethanol, and the latter 3 times as toxic as methanol. In his next report (1905), however, he stated that ethanol was only twice as toxic as methanol and that this rule held true for other alcohols up to and including octyl alcohol. Führer's final conclusions from his investigations were as follows: »In der homologen Reihe der einwertigen gesättigten primären Alkohole nimmt die Wirksamkeit für die normalen Glieder (Glieder mit unverzweigter Kette) um ein Konstant zu: Jedes folgende Glied ist dreimal so wirksam als das vorgehende. Die Glieder mit verzweigter Kette und die sekundären Alkohole sind weniger wirksam als die erstgenannten.»

The above statement does not entirely hold good as regards the warm-blooded animals. For example Joffroy and Servoux (1895) and Baer (1898) experimented with alcohols on mice and discovered that the increase in toxicity of homologous series of alcohols was less than threefold. The experiments were performed with methyl, ethyl, propyl, butyl and amyl alcohols.

Macht (1921) investigated the lethal effect of the same alcohols on cats and was able to verify Führer's rule. He also reported the secondary propyl, butyl and amyl alcohols to be less toxic than the corresponding primary alcohols. He stressed that we must discriminate between the acute and delayed effects of alcohols, as can clearly be shown with the methyl and benzyl alcohols.

Weese (1928) also studied the effects of different alcohols upon mice. The mice were given oral doses of methyl, ethyl, n-propyl and isopropyl alcohols in 25 per cent aqueous solutions. His investigations proved that the mouse was not dissimilar to other laboratory animals as regards its sensitiveness to alcohol. The lethal dose of absolute alcohol varied from 5.5 to 7.0 mg. He also investigated the toxicity of alcohol vapours on mice and discovered that as far as unsaturated vapours were concerned their narcotic and toxic effects supported the results of Richardson's experiments. Saturated vapours, however, gave somewhat different results. On account of their varying saturation points n-butyl alcohol and isobutyl alcohol were less effective narcotics than propyl alcohol, whereas secondary and tertiary alcohols were stronger.

When the toxicity of alcohols is being determined the results depend on a great variety of factors. Apart from differences in methods and in experimental circumstances there are several factors connected with metabolism which exercise a decisive influence upon the results.

Consequently, in toxicity studies it is of great value to analyse the metabolism of the drug concerned. It is important to know the absorption rate of the drug, its diffusion into the surrounding tissues, its detoxication rate and whether toxic derivatives which may cause fatal symptoms afterwards are generated, as, for example, in methanol poisoning.

The different aspects of the metabolism of ethanol have been very widely elucidated in the literature. Within the limits of this work it is not possible to give a detailed account of the literature; only the most important investigations from the author's point of view will be briefly reviewed.

The correlation between the toxicity and the concentrations of ethanol in oral administration has been investigated by several authors. Widmark (1916) made a comprehensive study of ethanol concentrations in man and discovered that the concentration of the alcohol ingested had no notable effect on the blood alcohol level. The concentrations he used were between 4.36 and 43.6 per cent. An ethanol dose taken immediately after a meal produced a blood concentration which was 1/5 of the concentration produced by the same dose taken into an empty stomach. Widmark (1934) attributed this phenomenon to the retarded absorption of ethanol and a specific action of food on ethanol. Widmark suggested that proteins, especially amino acids, exercise a retarding influence upon the absorption of ethanol. Fats, carbohydrates and water do not possess such an influence.

Miles (1922) and Tigerstedt and Kallioinen (1923) stated that in man the blood ethanol concentration level rose the higher the more concentrated the solution of ethanol administered, when identical amounts of absolute ethanol were used.

Contrary results were obtained by Tuovinen (1926, 1930). He reported that after ingestion of ethanol the blood ethanol level showed variations even in one subject under standardized conditions, and that dilute solutions raised the concentration in the blood more than strong solutions. He made his studies on 5

persons, each of whom received an ethanol dose of 60 ml. in 5, 20, 40 and 60 per cent dilutions. One of the persons was given 40 ml. of ethanol in four successive tests. Among other things Tuovinen paid attention to the inhibitory influence of food, especially proteins, upon the rise of the concentration in the blood. Schmidt (1937) reported that food retarded the rise of the blood ethanol level by 16—20 per cent.

Mellanby (1919) investigated ethanol concentrations in dogs and found that the blood concentration was the higher the stronger the ethanol solution was in the stomach. He also observed the inhibitory influence of food taken simultaneously.

Latven and Molitor (1939) gave mice ethanol in 70 and 95 per cent solutions orally, subcutaneously and intraperitoneally. They came to the conclusion that for each channel of administration the 95 per cent solution was slightly more toxic than the weaker solution (See Table 3). Hiestand *et al.* (1952) also compared the toxicity of different ethanol dilutions when administered intraperitoneally to mice. Their results are to be seen in Fig. 5 which reveals a marked increase in toxicity as the ethanol dilution increases from 12 to 60 per cent, while in concentrations above 60 per cent the toxicity declines, though in a small degree. As an explanation for this they suggested that strong ethanol solutions

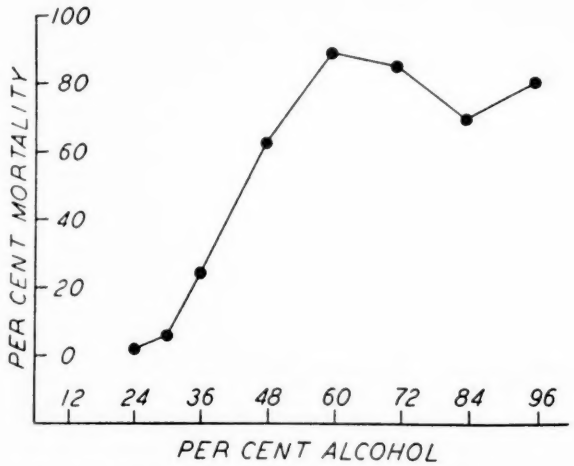


Fig. 5. — Effect of dilution of ethanol on toxicity. Toxicity was measured by the number of animals dead 24 hours after intraperitoneal injection of ethanol. 50 male mice were used in each dilution. All animals received the same amount of ethanol, differing only in dilution. (Hiestand *et al.* 1952)

»coagulate» the peritoneum and thus reduce the absorption of ethanol. Similar results were obtained by Aston and Cullumbine (1959). They found the LD_{50} for a 50 per cent ethanol solution in mice to be 3745 mg. per kg. using intraperitoneal administration, while for a 30 per cent dilution the LD_{50} was 6093 mg. per kg. This difference was attributed to the faster absorption of ethanol with the higher concentration. They calculated the absorption rate of the 50 per cent dilution to be 1.6 times quicker than that of the 30 per cent solution.

Through his experiments on rats Rasmussen (1940) proved that a strong oral ethanol concentration produced a weaker blood concentration than the same amount of ethanol administered in a weaker solution. Concentrations ranging from 7 to 14 per cent were called weak, and in later experiments the same result was obtained with dilutions between 4 and 6 per cent. Solutions of 21—35 per cent were regarded as strong concentrations. Dybing and Rasmussen (1940) came to the conclusion that this result was due to the specific histological structure of the mucous membrane of the rat's stomach.

To summarize: The contradictory results reported by different investigators seem largely to be due to the great variety of the concentrations used, but also to differences in the experimental circumstances, to different laboratory animals, and to methods of administration. From the investigations mentioned above we may, however, conclude that in man orally-given strong concentrations are absorbed more slowly and thus produce relatively lower blood concentrations of ethanol than corresponding weak solutions (Tuovinen). This result was not revealed clearly enough by the relatively weak dilutions used in earlier experiments (Widmark, Miles). A general conclusion drawn from experiments on animals was a positive correlation between a rise in the ethanol concentration and its toxicity. Contradictory opinions were offered by Hiestand *et al.*, who reported as a result of their experiments on rats that the toxicity of intraperitoneal doses stronger than 60 per cent did not increase in proportion to doses under 60 per cent. In oral experiments the rat differed from other laboratory animals for anatomical reasons (Dybing and Rasmussen).

The first investigations of the diffusion and detoxication of ethanol in the organism were published over one hundred years

ago. Since then numerous investigations and studies have been published. Gréhant (1896) is actually considered to be one of the founders of modern alcohol research. He investigated the distribution of ethanol and introduced the principle of the alcohol curve. He observed that for a time after administration the blood concentration of ethanol remained unchanged, at Gréhant's level.

When studying the elimination of ethanol in the organism Pringsheim (1908) came to the conclusion that most of it was oxidized in the liver. Similar results were obtained by Batelli and Stern (1910).

Carpenter (1929) studied the spreading of ethanol in the various tissues of hens. He administered ethanol by inhalation and made the important discovery that the ethanol concentrations were highest in the blood and lowest in the fat and bones.

A successful investigation of the distribution of ethanol in the organism was carried out by Nicloux (1931 a, b). He was able to find almost the whole amount of ethanol in the blood and tissues. He concluded that the blood concentration was dependent on the amount administered.

One of Widmark's (1932) greatest achievements in his alcohol research was his investigation of the oxidation rate of ethanol and of its diffusion in the organism. During the elimination phase there is a linear decrease in the blood concentration of ethanol, and its oxidation rate is independent of the concentration.

Widmark was followed by several investigators who devoted their time to the study of the oxidation problem of ethanol. Among others there were Haggard and Greenberg (1934), who gave their test animals intravenous injections of ethanol and came to the conclusion that the metabolism of ethanol is an exponential reaction, and that the oxidation speed of ethanol is directly proportional to its concentration. Their results were supported by Newman *et al.* (1937). Widmark, again, was confirmed by Neymark (1936) and Goldberg (1943). Jokipii (1951) reported that after the administration of ethanol (0.5 gm. per kg. in a 40 per cent aqueous solution) the ethanol content of the blood rose quickly and reached its maximum in 40 minutes. Ethanol was eliminated at an even rate.

Later investigators have been able to make use of more exact methods to shed light on the metabolism of ethanol. Casier (1954) administered C^{14} labelled ethanol to mice. His experiments revealed

that 90—92 per cent of the ethanol injected intraperitoneally was metabolized into carbon dioxide. This process took place mainly during the first five hours, after which period there was a progressive decrease till the sixteenth hour. During the first seven hours most of the free ethanol was burned; between the seventh and the sixteenth hours the ethanol fixed in the tissues was also metabolized. Secondly, his experiments revealed that the amount of ethanol in mice was maximal 30—60 minutes after the injection. The greatest amounts of ethanol were to be found one hour after the injection in the liver, where most of the metabolism took place. Only 1.2 per cent of the ethanol was excreted in the urine.

The rate of elimination of ethanol in mice has been studied by Marshall and Owens (1955). They found the rate of oxidation of ethanol to be twice as great during the first hour as later. After this high initial rate oxidation took place more slowly and rectilinearly. They used a dose of 4 gm. per kg. (Fig. 6).

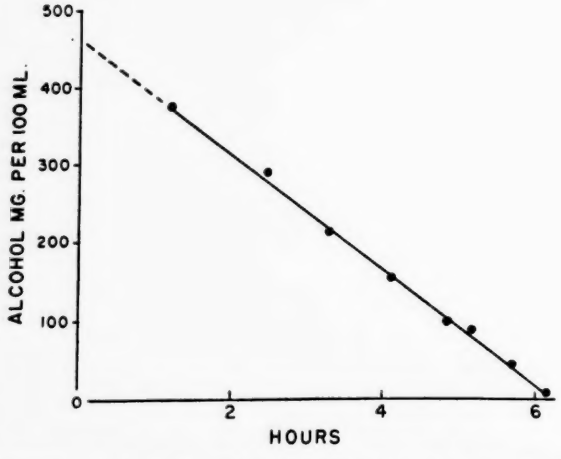


Fig. 6. — Concentration of ethanol in the tail blood of one mouse determined at various times after intraperitoneal injection of 4 gm. of ethanol per kg. A straight line has been fitted to the values plotted arithmetically. (Marshall and Owens 1955)

Nelson *et al.* (1957), when checking the above experiment, came to the conclusion that during the first three hours after administration of ethanol the metabolism was a linear one. They also found smaller mice to have higher metabolism rates than

bigger ones. An interesting observation is the one made by Kinard *et al.* (1959) of the individual differences of metabolism in rats. These differences were found to be due to differences in the size of the liver; a rat with a large liver was able to eliminate ethanol in a shorter time than one with a small liver.

2. BARBITURATES

After the first barbiturate, barbital (Veronal), had been announced in 1903 by Fischer and Mering (Goodman and Gilman 1941), an extensive literature concerning the chemistry, pharmacology and clinical use of barbiturates was published. The enormous development of biochemistry, analytical chemistry and other basic sciences during the last decades has greatly elucidated and widened our knowledge of the distribution of these drugs in the organism, their metabolism and their modes of action.

It is not the purpose of this study to go into all the details of the problems concerning the metabolism of barbiturates. The metabolism of the ultra short acting barbiturates used, hexobarbital and thiopental, has been dealt with to the extent considered necessary.

Jailer and Goldbaum (1946) investigated the concentration of thiopental in the plasma and tissues, using a spectrophotometric method. The concentration in the plasma reached its maximum rapidly after an intravenous injection, and there was a clear correlation between the depth of anaesthesia and the plasma concentration. Thiopental disappeared quickly from the tissues; only a small amount was excreted in the urine and very little was stored in the tissues.

Goldbaum and Smith (1948) studied the protein binding of the barbiturates in the blood and found that it was dependant on the pH of the blood. The thiobarbiturates were bound to a larger extent than the others; for example, the binding percentage of thiopental was 65, whereas that of hexobarbital was 29. The binding was also inversely proportional to the plasma concentration.

Bollman *et al.* (1950) studied the distribution of thiopental in the different tissues of rats. They used S_{35} labelled thiopental. 30 seconds after intravenous injection most of the thiopental had

disappeared from the blood and was fairly evenly distributed in the different organs. Thiopental was not stored in any special organ, and its concentration diminished evenly in each. After one hour about half of the maximum amount of the thiopental in an organ was still found, when the dose was 40 mg. per kg.

Thiopental was fairly evenly distributed between the different parts of the brain, but in cases of high plasma concentrations (over 1.7 mg. per 100 ml.) the concentration in the thalamus was 2 to 2½ times as high as in the lumbar spinal cord. (Hubbard and Goldbaum 1950, Brodie *et al.* 1952, Mark *et al.* 1957).

According to Bollman *et al.* (1950) thiopental is not metabolized in any special organ. Brodie *et al.* (1950) reported that the complete metabolism of thiopental in the human organism is a slow procedure, the rate of metabolism being about 15 per cent an hour. The quick decrease of the plasma level probably depends on the fact that major part of the thiopental accumulates in the fat. Later on (1952) Brodie *et al.* confirmed this statement and reported that the short time of action of thiopental is not due to a quick rate of metabolism — it is, in fact, slow — but to the drug's accumulation in the fat. The ultra short acting barbiturates need 1—2 days to be completely eliminated (Brodie *et al.* 1950).

According to Stolman and Stewart (1960) the barbiturates disappear from the blood at a rate which depends on their type and the dose given, but in general it is rapid. Intravenous doses give an immediately high plasma concentration which falls rapidly until equilibrium with the major tissues and fluid compartments is reached. The explanation may be this: the amount of intracellular and extracellular fluid is about ten times that of the plasma; the barbiturates obviously penetrate easily into the cells; in consequence it is easy to understand that within a few minutes after an intravenous injection the drug concentration of the plasma has diminished to a tenth or less of the original amount.

There are numerous experimental studies of *the combined effects of ethanol and the barbiturates*. Their great number is largely due to the increasing use of the barbiturates, which has made this problem clinically most important. The results of the investigations have often proved contradictory, and this is another factor which deepens interest in study of the combined effects.

The first to investigate the effects of ethanol and phenobarbital

(Gardenal) were Carrière *et al.* (1934). One of their 5 rabbits was given 2 ml. of 30 per cent ethanol, with the result that it developed phenobarbital coma later than the rabbits that had not received ethanol. In other experiments animals in a phenobarbital coma were given injections of 2 ml. of 30 per cent ethanol every hour, with the result that the coma had already disappeared after 5—10 hours. The investigators give no statement about the duration of the coma in the animals that did not receive ethanol. On the basis of these experiments they considered ethanol and phenobarbital to be antagonistic.

The results of Carrière *et al.* were tested by Allegri (1935). He first gave his rabbits phenobarbital; after the stabilization of coma he gave 1 ml. of 30 per cent ethanol per kg. every hour. His results only supported Carrière's findings in those cases where the dose of phenobarbital very slightly exceeded the lethal dose, 0.10—0.15 gm. per kg. In dog experiments all the animals died from coma in spite of the administration of ethanol. Similarly, in experiments with guinea-pigs all the animals died after receiving the lethal dose of barbital sodium (Veronal sodium) and ethanol. The results were also negative when experiments were performed with barbital sodium on rabbits. Allegri concluded that coma produced by phenobarbital or barbital sodium cannot be remedied by the administration of ethanol.

In her investigations Olszycka (1935, 1936) obtained results quite contradictory to those of Carrière *et al.* She first determined the sleeping time for rats and mice after the administration of ethanol or butethal (Neonal). The rats were given ethanol in doses of 1.6, 2.1 and 3.2 mg. per gm., together with butethal in doses of 0.04—0.065, 0.03—0.15, 0.03—0.09 mg. per gm. Olszycka used a special potentiation coefficient, defined by dividing the effect of the combined administration by the sum of the individual effects. The greatest potentiation was produced by the combination 3.2 mg. of ethanol per gm. and 0.03, 0.04, 0.05, 0.06 or 0.065 mg. of butethal per gm. The coefficients of potentiation for these doses were 14.5, 23.5, 40.0, 56.0 and 11.9 respectively. Here is an example to illustrate the combined effects. The animal received an ethanol dose of a strength to produce a sleep of 2—3 minutes; together with this a dose of butethal which alone would produce a sleep of 11 minutes was administered; the animal slept 191 minutes. She

concluded that as far as the duration of sleeping time is concerned there exists a potentiation between butethal and ethanol. Moreover, she reported the potentiation to be greater with smaller doses.

Dille *et al.* (1935) injected the carotid arteries of dogs with barbital dissolved in 50 per cent solution of methanol. They found the concentration of barbital to be always higher in the brain than in the other tissues. After the injection of barbital or barbital sodium dissolved in water, the amount retained by the brain was always smaller than the amounts retained by the liver or by the kidneys. After injecting water or methanol solutions into other arteries or veins the concentration in the brain was never higher than in the other tissues. They concluded that, when injected direct into the carotid artery, methanol enables the brain tissue to retain greater amounts of barbital.

Olszycka's coefficient was used by Dille and Ahlquist (1937) for determining the combined effect of pentobarbital and ethanol on rabbits. The drugs were given intravenously, and the effect was checked by the duration of the sleeping time, as indicated by the absence of the righting reflex. This coefficient cannot, however, be employed if one or both of the doses are below the threshold level for sleep. They also determined the rate of elimination of ethanol and pentobarbital from the blood and found the rate for pentobarbital to be practically unaffected by the presence of ethanol. They concluded that a potentiation between ethanol and pentobarbital existed and that this potentiation was greater with small doses. This conclusion supports the experimental results of Olszycka, Dille and Ahlquist. Contrary to Dille's and Ahlquist's reference, Allegri did not confirm the results of Carrière *et al.*

Jetter and McLean (1943) gave rats ethanol intraperitoneally and phenobarbital subcutaneously. After the maximal sublethal dose had been determined for each drug separately, they examined their combined effect. The maximal separate doses that left the animals alive were 8 mg. of ethanol per gm. diluted in 20 per cent sodium chloride, and 0.20 mg. of phenobarbital per gm. diluted in 0.9 per cent sodium chloride. Both of these doses were then administered in combination to a small group of rats. A group of 6 animals received a combination of 50 per cent of these sublethal doses. One of the animals died. The investigators concluded that the combined administration of these doses produced the same

effect as the whole sublethal dose of either ethanol or phenobarbital. They further concluded that between phenobarbital and ethanol there existed a marked synergistic effect. The maximal lethal dose of each drug caused death without exception. The maximal lethal dose of one drug in combination with half of the maximal lethal dose of the other drug produced death in most animals. Half of the sublethal doses of each drug in combination produced deep narcosis with occasional fatalities.

Seeberg and Dille (1943) demonstrated through experiments on a cat that barbital is more effective as an elixir for its ethanol content than when administered alone.

Ramsey and Haag (1946) conducted an experimental study on mice to find out the combined effect of secobarbital sodium (Seconal), pentobarbital and barbital in combination with ethanol. They determined the LD_{50} of each barbiturate and ethanol for oral administration. The animals were then given 70 per cent of the LD_{50} of each barbiturate together with 4.2 ml. of 95 per cent ethanol, the LD_{50} of which was 11.2 ml. per kg. In each case ethanol increased the toxicity of the barbiturate, calculated according to the mortality percentage. This investigation also showed that ethanol had a greater influence on the toxicity of secobarbital sodium. They also studied the combined effect of the intravenous administration of thiopental and the oral administration of ethanol on dogs. The anaesthetic dose of thiopental, 10.9 mg. per kg., decreased to 6.9 mg. per kg. if the animal had earlier received 1.5 ml. of 95 per cent ethanol per kg. Ethanol also prolonged the duration of anaesthesia to a marked degree. The duration of the anaesthesia was checked by the cornea reflex and the return of the voluntary movements. The influence of ethanol on the lethal dose of thiopental was also examined in dogs. The lethal dose of thiopental alone was determined as 68.7 mg. per kg. when intravenously administered. The lethal dose for dogs under the influence of ethanol was 40.6 mg. per kg. Ethanol caused no noteworthy changes in the anaesthetic doses of thiopental even though it was given only one, two, or three hours before the administration of thiopental. On the other hand, the duration of the anaesthesia for a fixed amount of thiopental was longest when the thiopental was given one hour after the administration of ethanol, and shortest when given three hours after it. In con-

formity with Carrière *et al.* (1934) the onset of barbital anaesthesia seemed to be delayed by the influence of ethanol, and the duration of the anaesthesia was also prolonged. The absorption and elimination of the ethanol was not dependent upon the barbital nor were there any changes in the ethanol content of the blood due to the administration of barbital. In their investigation Ramsey and Haag also came to the conclusion that picrotoxin lost some of its effect as an antidote in pentobarbital poisonings if ethanol were given simultaneously. When measuring the effect by the duration of the anaesthesia, they obtained the result that the picrotoxin experiments proved the combined effect of barbiturates and ethanol to be potentiative and not only additive.

Sandberg (1951) gave six short acting barbiturates (thialbarbitone, hexobarbital, cyclobarbital, amobarbital, thiopental, and 1-carbethoxymethyl-5,5-diallylbarbituric acid) to mice; he gave ethanol as a vapour. There were changes in the degree of potentiation when different barbiturates were combined with ethanol. A particular coefficient was used for measuring potentiation. Sandberg presumed the anaesthetic effect of the barbiturates to be inversely proportional to the logarithmic value of the time of induction, and he introduced the following equation:

$$C = \frac{1}{\dfrac{\log \text{ time (ethanol + barbiturate)}}{\dfrac{1}{\log \text{ time (ethanol)}} + \dfrac{1}{\log \text{ time (barbiturate)}}}}$$

The lowest value for the coefficient was 0.5, which indicated that no potentiation existed. The coefficient had no theoretical upper limit. Sandberg's investigations proved the existence of a potentiative type of synergism between the actions of ethanol and each of the six barbiturates. Hexobarbital had the strongest effect and cyclobarbital the weakest. The effectiveness of the potentiation was influenced by the type of barbiturates used and by the size of the dose.

Smith and Loomis (1951) observed that the intravenous administration of 30 mg. per kg. of thiopental shortened the reaction time for pain in mice. But there was no noticeable change after

intravenous administration of 1 gm. per kg. of ethanol, either alone or in combination with thiopental. They also compared the duration of anaesthesia in mice, rabbits and dogs after intravenous administration of thiopental both alone and in combination with ethanol. The duration of anaesthesia after combined administration was found to be longer than the sum of the individual periods. This is evidence of possible synergism after combined administration of ethanol and thiopental. They declared that a competition between these two drugs for the same or the same kind of enzyme system may take place, and this may be why the metabolism of thiopental is retarded by ethanol.

Apart from Carrière and his collaborators all the investigators mentioned above found the combined action of ethanol and barbiturates to be greater than the sum of the actions of the individual drugs. This phenomenon is called potentiation by some investigators and a potentiative kind of synergism by others.

Fearn and Hodges (1953) made an experimental study of the combined action of amobarbital (Amytal) and ethanol on mice. The first series of experiments was performed in order to find out the influence of ethanol on the acute toxicity of amobarbital. 300 mice were used in these experiments. Amobarbital was administered as a solution containing 20 mg. of the drug per ml. and ethanol in a 50 per cent aqueous solution. Both were administered orally. First the MLD (median lethal dose) was determined for each individual drug; then the doses of ethanol were ascertained which were needed to kill 50 per cent of the mice after they had received $^1/_4$, $^1/_2$ and $^3/_4$ of the MLD of amobarbital. The second series of experiments was made on 150 mice to elucidate the anaesthetic effect of amobarbital and ethanol. The investigators determined the MED (median effective dose) which produced a sleep of one hour at least. The righting reflex was used as the criterion. The animals were placed on their backs and those that did not get up within an hour after the intraperitoneal administration of the drug were considered anaesthetized. The results were presented diagrammatically according to Gaddum. (Fig. 7).

Fearn and Hodges proved that amobarbital and ethanol were synergistic and that this synergism was simply additive. During the investigation ethanol was never found to have a potentiative influence on the toxic or anaesthetic action of amobarbital.

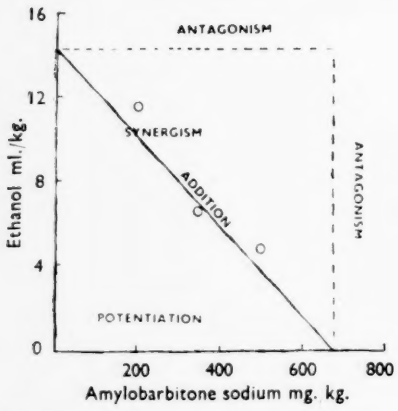
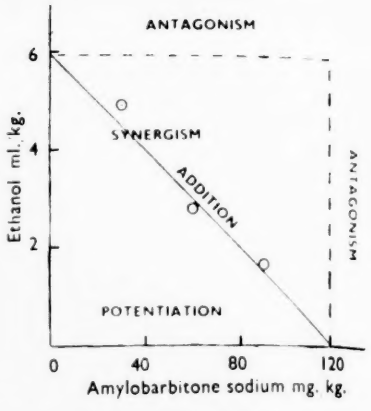


Fig. 7. — Doses of amobarbital sodium and ethanol necessary to produce 50 per cent mortality in mice.



Doses of amobarbital sodium and ethanol necessary to maintain anaesthesia in 50 per cent of the mice for at least one hour.

(Fearn and Hodges 1953)

Gruber (1955) tried to find theoretical grounds for the additive and potentiating effects of two agents with special reference to ethanol and barbiturates. He defined synergism as the combined effect of two drugs when their combined effect is greater than the sum of their individual effects. He experimented on mice; the righting reflex was used as the criterion for the sleeping time; death up to 12 hours after the administration of the drugs was used as the criterion for the mortality rate. Ethanol, secobarbital and phenobarbital were administered to the mice intraperitoneally diluted in 0.9 per cent sodium chloride, the dose being 0.5 ml. per 20 gm. of weight. The experiments with secobarbital and ethanol, as well as those with phenobarbital and ethanol, gave no evidence of potentiation.

Gruber scrutinized the earlier investigations of the combined effects of ethanol and barbiturates and asserted that none of them gave convincing evidence of the existence of potentiation. He also stated that the final results may have been influenced by several factors occurring during the progress of such an investigation. Thus, the absorption of either or both constituents may be accelerated by some factor; the diffusion of either or both drugs in the surrounding organism may be changed; or the deactivation and the excretion of the drugs may be retarded or speeded up.

Erroneous results may also be due to differences in the duration of the effects, in the starting times, and in the durations of the activities of the constituents. His final conclusion was that the combined action of ethanol and barbiturates can be nothing but additive.

Archer (1956) investigated the combined effects of ethanol and secobarbital. He determined the LD_{50} of each drug for mice, and used intraperitoneal injection. Secobarbital was administered in an aqueous solution with a concentration of 1 ml. per 100 gm. of weight; the ethanol was given in a 20 per cent aqueous solution. Half of the LD_{50} of each drug was then administered to 30 mice; the results proved that the combined action of ethanol and secobarbital was additive and that this addition was less than the total summation of the actions of the constituents.

In their experimental studies on mice Aston and Cullumbine (1959) also determined the LD_{50} for the intraperitoneal administration of 30 per cent and 50 per cent ethanol, for secobarbital sodium, for phenobarbital, and for the intravenous administration of 30 per cent ethanol. They observed that the toxicity of ethanol in mice, when given intraperitoneally, depended on its concentration. Secobarbital sodium and phenobarbital sodium when administered in combination with ethanol revealed an additive action (a negatively correlated independent additive joint action). This result was supported by experiments performed on rabbits with intravenous injections of ethanol and pentobarbital sodium, which were found to act additively.

The combined action of ethanol and pentobarbital on mice was investigated by Graham (1959). Ethanol was administered in a 40 per cent aqueous solution; pentobarbital was administered in various concentrations but always in the same volume, 10 ml. per kg. The LD_{50} of pentobarbital was then determined after the mice had been given, one hour earlier, ethanol 25, 50, or 75 per cent of its LD_{50}. Graham reported ethanol and the barbiturates to exercise a depressing additive effect on the central nervous system (Fig. 8).

Through his experiments on rats Graham also tried to elucidate the influence of ethanol on the absorption of the barbiturates. As far as ethanol was concerned he could not confirm the observation of Dille *et al.* (1935). They had declared that a dose of 50 per cent

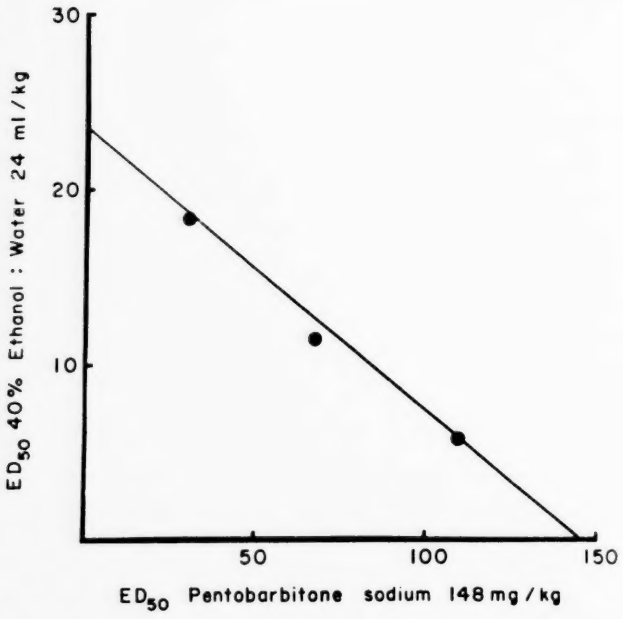


Fig. 8. — The additive interaction of 40 vol. per cent ethanol: water and of pentobarbital sodium when both are given to mice by stomach tube. The relation is a simple additive one. (Graham 1960)

methanol injected into the carotid artery of a dog increased the content of the barbiturate in the brain tissue. In Graham's experiments ethanol did not increase the absorption of the barbiturate, nor was the brain concentration dependent upon the dilution of ethanol given to the rat.

3. MORPHINE

According to Joël's report (1928) subcutaneous morphine doses of 0.5 mg. per gm. produced such violent convulsions in mice that the animals died. Joël also observed the effects of smaller doses and distinguished between three typical syndromes: The first was the so-called Straub's syndrome (Hermann 1912), in which the tail of the mouse stood up erect or, with larger doses, bent in a stiff curve over the animal's head. Immediately after its disappearance the sign could be restored by irritating the animal — by acoustic stimuli, for example. The second syndrome was characterized by peculiar postures, so that a sitting mouse resembled a shock of corn or ran about with a crooked back. In the third

syndrome the mouse ran in a circle under a glass bell. This sign is called the manège phenomenon. The animal also responded readily to sounds and noise.

With subcutaneous administration the lethal dose for the mouse was determined by Schmitz (1926) as 1.2 mg. per gm. This was considerably larger than the lethal doses determined by other investigators. The great variations in the lethal doses were attributed by Schmitz to several factors, such as differences in breed, food and outer experimental conditions.

Matsuda and Yamaguti (1940) determined the lethal morphine doses for mice in the cases of different channels of administration: intravenous, intraperitoneal, and subcutaneous. The intravenous dose was injected via the vein of the tail. If the mouse died soon after the injection, they attributed the death to respiratory distress. If the mouse remained alive for a few hours after the injection, it got several attacks of cramp, from which t finall y died. This led the authors to think that the slower death may have been caused by some convulsant substance produced by the metabolism of morphine. A substance like this was found in the frog. Matsuda and Yamaguti determined the LD_{50} as 0.31 mg. per gm. for the mice which died from respiratory distress. For intraperitoneal administration it was 0.45 mg. per gm. and for subcutaneous administration 0.55 mg. per gm. (Figs. 2, 3).

The metabolism of morphine has been studied by several investigators both in animals and in man. Through Gross and Thompson's (1940) experiments on dogs and through Oberst's (1940) experiments on man it was demonstrated that morphine is excreted in the urine not only in a free form but also in a combined form, possibly in combination with glucuronic acid. Their discovery was confirmed by Seibert *et al.* (1954).

When man was given a therapeutic dose of radioactive morphine, most of it was excreted in the urine during 24 hours, 7—10 per cent in the faeces, and 3.5—6 per cent through respiration as CO_2 (Elliott *et al.* 1954). March and Elliott (1954) carried out experiments with radioactive morphine (morphine-N methyl C^{14}) on rats. Most of the morphine was excreted in the urine and into the intestinal canal; only small amounts were found in the central nervous system. The exhailed air of the male mice contained more of the radioactive $C^{14}O_2$ than that of the females.

The analgetic and sedative synergism of the combination morphine-scopolamine is a well-known example of the combined action of morphine and another drug. Bürgi (1916) regarded it as a model example of potentiation. Turpeinen (1944) proved through his experiments that when morphine in combination with atropine (8—12 mg. of morphine and 0.10—0.15 mg. of atropine) or in combination with scopolamine (16—20 mg. of morphine and 0.48—0.68 mg. of scopolamine) was given as a premedication prior to ether narcosis, the amount of ether used for anesthesia was markedly smaller than the amount of ether needed for patients anaesthetized without premedication. He further discovered that premedication with morphine-scopolamine saved 40 per cent more ether than that with morphine-atropine. Turpeinen considered that the action of the combination morphine-scopolamine in ether narcosis was of the potentiative type.

The combined action of morphine and atropine will be discussed in connection with atropine.

Eerola *et al.* (1955) investigated the combined action of ethanol and morphine on mice; Venho *et al.* (1955) investigated the same action on mice habituated to ethanol. On the basis of these investigations the combined action of morphine and ethanol was of the potentiative type.

Wagner and Wagner (1958) made an experimental study on mice to analyze the combined action of ethanol and Polamidon (dl-dimethylamino-4.4diphenylheptanon (5) hydrochloride). The latter drug is a synthetic substance, the effect of which is similar to that of morphine. They determined the LD_{50} of each drug for subcutaneous administration. The LD_{50} of ethanol was 8442 ± 666 mg. per kg., and that of Polamidon 54.5 ± 6.5 mg. per kg. Thereafter three different series of experiments were performed: 1. The test animals were given 4019 ± 160 mg. per kg. of ethanol, and 10 minutes later 25.31 ± 1.6 mg. per kg. of Polamidon. 2. The animals received 6138 mg. per kg. of ethanol and 11.5 mg. per kg. of Polamidon. 3. The animals in the third group were given 5013 mg. per kg. of ethanol and 11.5 mg. per kg. of Polamidon. The intention was to find out whether the combined action of these two drugs was of the potentiative or the additive synergistic type. The results were represented graphically according to Gaddum's method. Although the points of the experimental results fell within the potentiative triangle in all three series, the

investigators did not consider that a distinct potentiation existed between ethanol and Polamidon; it was merely an additive synergism.

Clinical observations of the combined action of ethanol and morphine were made, as mentioned above, by Møller (1952) and Naalsund (1955). According to their reports the depressive effect of ethanol was strongly potentiated by morphine.

4. ATROPINE

According to Joël (1928) the lethal dose of atropine when given subcutaneously to mice was 0.5 mg. per kg. The animals died rather late, often as late as 24 hours after the injection. As a rule, mice were much more sensitive to atropine than other laboratory animals (Evertsbusch and Geiling 1955).

The metabolism of atropine has been investigated by Evertsbusch and Geiling among others; they used radioactive atropine. They reported as follows: Immediately after subcutaneous injection the atropine spread all over the organism except for the central nervous system. After one hour the blood was purified from atropine, which was concentrated in the liver, the kidneys and the lungs. The liver secreted it into the bile, from which it was again absorbed into the enterohepatic blood circulation through the intestine. 85 per cent of the atropine administered was excreted in the urine, 13 per cent in the excrement, and 1.5 per cent in the air, exhaled as CO_2.

Gosselin *et al.* (1955) also used radioactive atropine and tropic acid in their mouse experiments. They demonstrated that 80—90 per cent of the atropine was excreted in the urine. The highest atropine concentrations were found in the gall bladder, the urinary tract, the intestinal canal and the liver; the lowest concentrations were in the suprarenal glands and the lungs.

In the literature there are no earlier investigations of the combined action of atropine and ethanol on mice.

Several investigators have made experiments on mice and other animals in order to elucidate the combined action of atropine and morphine. Many investigations revealed antagonism between these two drugs (Heubach 1877, Binz 1887, and Bashford 1901).

It should be observed, however, that the first two did not use lethal doses of morphine in their experiments. Bashford, on the other hand, attained positive results when giving atropine to rats after lethal doses of morphine. Several other investigators, however, arrived at contradictory results. Lenharz (1887) and Brauer (1921) concluded from their clinical experience and from their experimental studies that atropine is not antagonistic to morphine.

Joël (1928) made extensive experiments on rabbits, rats and mice in order to investigate, among other things, the combined action of atropine and morphine. He reported that irrespective of the dosage, of the channel or the order of administration, atropine may strongly enhance the effect of morphine. This enhancing effect was, however, small in mice.

Friedberg (1931) investigated the effect of atropine on mice which had been given sedatives beforehand. He divided his centrally depressive drugs into two groups: the brain-stem sedatives, including phenobarbital and butallylonal, and the cerebral sedatives, including chloral hydrate and paraldehyde. He found that atropine alone produced no sedative symptoms in rabbits. He also used doses of sedatives which did not produce narcosis when administered separately. When administered intravenously in combination atropine and butallylonal made rabbits fall into a short but comparatively deep sleep. Experiments with phenobarbital gave the same result. Chloral hydrate, on the other hand, had only a very slightly enhancing effect. The action of paraldehyde was not affected by atropine at all.

Hendrych (1936) discovered in his experiments on mice that morphine-ether narcosis was deepened by atropine. The effect of atropine on the central nervous system was the same as on the peripheraln erves: paralysis. Hendrych thus disagreed with those earlier investigators who thought there was antagonism between atropine and morphine.

In experiments on mice Mensch and Jongh (1958) demonstrated that atropine (8—180 mMol. per kg.), when injected intraperitoneally 15 minutes before an intravenous dose of pentobarbital (20 mg. per kg.), had an enhancing effect on the barbiturate. This effect was strengthened by an increase in the atropine dose. The righting reflex was used as the criterion. They also investigated the combined action of atropine and ether on rats. These animals also

received an intraperitoneal injection of atropine 45 minutes before narcosis. Atropine was found to lengthen the period of ether narcosis. The sleeping time was controlled using the righting reflex.

5. SCOPOLAMINE

In order to create any effect by means of scopolamine, mice have to be given very large doses. Scopolamine is then no longer a sedative; we must speak of toxic doses. According to Molitor (1936) the LD_{50} given subcutaneously to mice is 1700 mg. per kg.

The absorption of scopolamine from the alimentary tract is very quick, and in the blood it is bound to proteins. The fate of scopolamine in the organism is not well known. Glick and Glaubach (1941) reported that it is hydrolyzed in the serum of the rabbit, but this is not true for rats, guinea-pigs or dogs. The excretion of scopolamine depends on the dose and the method of administration. For the rats 1.2 per cent of the scopolamine dose given orally is excreted in the urine in 3 days, 13 per cent is excreted when given subcutaneously and 18 per cent when the dose is intravenous (Tønnesen 1950).

No investigations can be found in the literature on the combined action of scopolamine and ethanol.

On the other hand, a considerable number of investigations have been published on the combined action of scopolamine and certain other drugs with a depressive effect on the central nervous system. After the publication of Schneiderlin's investigation (1903) on the enhancing effect of scopolamine upon morphine, several investigators have used scopolamine in their experiments and studied its combined action with sedatives. Hauckold (1910) investigated the combined action of scopolamine and urethan, Lewin (1916) that of scopolamine and chloralhydrate, and Bermann (1916) that of scopolamine and phenobarbital sodium. All these drug combinations produced in rabbits, on which scopolamine alone has little effect, a deeper narcosis than the sedative alone. Mehes (1929), too, used the combined action of bromides and scopolamine to anaesthetize rabbits. In Friedberg's (1931) experiments on rabbits scopolamine enhanced the effect of butallylonal, phenobarbital and to a small degree chloral hydrate, but not the effect of paraldehyde. Hendrych (1936) compared the enhancing effect of atropine with that of scopolamine

on ether narcosis in mice; the effects were found to be equally great.

Mensch and Jongh (1958) discovered that scopolamine doses of 1—64 mMol. per kg. considerably strengthened the effect of pentobarbital upon mice; but scopolamine differed from atropine in its maximal effect, which was created with a dose of 16 mMol. per kg. only. The same investigators (1959) found that scopolamine prolonged the duration of ether narcosis in mice 12 times as much as atropine.

IV. THE PRESENT INVESTIGATION

A. MATERIAL AND METHODS

White male mice, all of the same strain, bred in the colony of Mankkaa, were used for the experiments. The animals were 18—37 gm. in weight, most of them between 20 and 30 gm. They were fed on a standard diet (Table 4) and the external circumstances were kept as constant as possible (the room temperature 20—25°C). The mice were starved 2 hours before the experiments. Each mouse was used for one experiment only. During the experiment the mouse was kept in a glass jar, the bottom of which was covered with a thin layer of sawdust. The mice were weighed immediately before the experiments to an accuracy of 1 gm. In some series, with the weight of the mice varying between 18 and 22 gm., the accuracy was 0.5 gm.

TABLE 4

DIET OF THE MICE USED IN THE EXPERIMENTS

Dried liver	10	per cent
Gelatin	0.5	» »
Fish meal	6	» »
Meat meal	2	» »
Soy grits	3.5	» »
Sunflower grits	2.7	» »
Corn meal	24	» »
Oat meal	10.5	» »
Barley meal	9.5	» »
Wheat meal	9.5	» »
Wheat bran	12.5	» »
Lucerne meal	2.2	» »
Wheat germs	2	» »
Yeast	2	» »
Lime	1	» »
Phosphate	1	» »

Choline	0.130 per cent
Sodium chloride	0.815 » »
Magnesium sulphate	0.025 » »
Iron sulphate	0.025 » »
Cobalt sulphate	0.002 » »
Copper sulphate	0.002 » »
Iodine calcium	0.001 » »

Vitamin A	5000 i.u. per kg.
Aneurin chloride	4 mg. per kg.
Riboflavin	13 mg. per kg.
Niacinamide	10 mg. per kg.
Pyridoxine	5 mg. per kg.
Biotine	0.2 mg. per kg.
Pteroylglutamic acid	1 mg. per kg.
Calcium pantothenate	10 mg. per kg.
Vitamin D_3	1000 i.u. per kg.

The ethanol was given as an aqueous solution in a concentration of 20—30 vol. per cent, which was prepared immediately before the experiments. The solution was given with a cannula through the mouth direct into the stomach, after Behrens' method (Figs. 9—11). The cannula was a specially made, 8 cm. long injection needle, 0.75 mm. in diameter; its point was shielded with silver plating to facilitate the introduction. The size of the syringe used was 0.5—1 ml. The introduction was made with the cannula

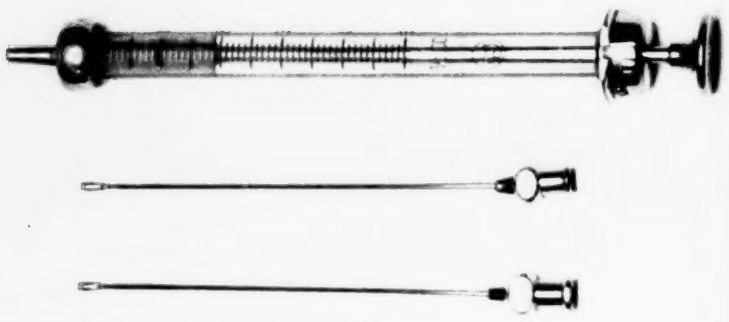


Fig. 9. — The syringe and the cannula used for the injection of ethanol.

Fig. 10. — Introduction of the cannula into the stomach of the mouse.

Fig. 11. — X-ray film after the injection of the contrast medium into the stomach of the mouse.

fixed to the syringe, and the injection of the solution was made as soon as the cannula was in place. If part of the solution was wasted or other technical complications occurred, the mouse was rejected. In the combined experiments the ethanol was given first, followed by the second drug immediately after.

The other drugs used, hexobarbital (Evipan sodium, Bayer), thiopental (Intraval sodium, May & Baker), morphine hydrochloride, atropine sulphate and scopolamine bromide, were dissolved in distilled water immediately before the experiments.

The concentrations of the solutions were:

Hexobarbital	5.0	per cent
Thiopental	1.25— 2.5	» »
Morphine hydrochloride	2.5	» »
Atropine sulphate	5.0	» »
Scopolamine bromide	10.0	» »

The volume of the dose given did not exceed 0.5 ml. and the drugs were injected subcutaneously with a thin needle in the neck region of the mouse. The hole made by the needle in the skin was compressed with the fingers for a while to prevent leakage of the solution.

The criterion of toxicity was the death of the mouse, and the control time was 24 hours. All the dead mice were autopsied in order to exclude other causes of death and also technical complications. Altogether 2,900 mice were used for the experiments, including the preliminary tests and the control series.

B. RESULTS

1. EXPERIMENTS WITH ONE DRUG

Each drug to be tested was given to a group of 20 mice. The amounts were intended to be sufficient to produce death in some of the animals. The number of animals that died was registered. Through these observation figures or mortality points the lethality of the drug concerned was determined mathematically between 5 and 95 per cent (LD_5—LD_{95}). The number of these points fluctuated

to some extent in the different series of experiments, depending upon the more limited or the more extensive dispersion of the points.

Ethanol. Six points were determined. The mortality percentage fluctuated between 10 and 95. LD_{50} was 9.5 ml. per kg. Table 5.

TABLE 5

ETHANOL

Dose ml/kg	log x	Number of animals	Number of deaths	Mortality percentage
7	0.845	20	2	10
8	0.903	20	4	20
9	0.954	20	8	40
10	1.000	20	9	45
11	1.041	20	15	75
12	1.079	20	19	95

Hexobarbital. Eight points were determined. The mortality percentage fluctuated between 0 and 95. LD_{50} was 280 mg. per kg. Table 6.

TABLE 6

HEXOBARBITAL

Dose mg/kg	log x	Number of animals	Number of deaths	Mortality percentage
200	2.301	20	0	0
230	2.362	20	3	15
250	2.398	20	4	20
270	2.431	20	8	40
300	2.477	20	12	60
320	2.505	20	16	80
350	2.544	20	18	90
370	2.568	20	19	95

Thiopental. Seven points were determined. The mortality percentage fluctuated between 0 and 80. LD_{50} was 100 mg. per kg. Table 7.

TABLE 7

THIOPENTAL

Dose mg/kg	log x	Number of animals	Number of deaths	Mortality percentage
60	1.778	20	0	0
70	1.845	20	1	5
80	1.903	20	5	25
90	1.945	20	6	30
100	2.000	20	9	45
110	2.041	20	13	65
120	2.079	20	16	80

Morphine. Six points were determined. The mortality percentage fluctuated between 10 and 90. LD_{50} was 300 mg. per kg. Table 8.

TABLE 8

MORPHINE

Dose mg/kg	log x	Number of animals	Number of deaths	Mortality percentage
100	2.000	20	2	10
200	2.301	20	5	25
300	2.477	20	10	50
400	2.602	20	15	75
500	2.699	20	16	80
600	2.778	20	18	90

Atropine. Six. points were determined. The mortality percentage fluctuated between 10 and 85. LD_{50} was 400 mg. per kg. Table 9.

TABLE 9

ATROPINE

Dose mg/kg	log x	Number of animals	Number of deaths	Mortality percentage
100	2.000	20	2	10
200	2.301	20	3	15
300	2.477	20	6	30
400	2.602	20	10	50
500	2.699	20	13	65
600	2.778	20	17	85

Scopolamine. Six points were determined. The mortality percentage fluctuated between 10 and 85. LD_{50} was 1100 mg. per kg. Table 10.

TABLE 10

SCOPOLAMINE

Dose mg/kg	log x	Number of animals	Number of deaths	Mortality percentage
800	2.903	20	2	10
900	2.954	20	4	20
1000	3.000	20	6	30
1200	3.079	20	11	55
1300	3.114	20	16	80
1400	3.146	20	17	85

2. EXPERIMENTS WITH TWO DRUGS

The primary component in these experiments was ethanol. The mortality points were determined by changing the concentrations of the drug used, the dose of one drug was kept constant while the concentration of the other one was changed; then the concentration of the first drug was changed while the second was kept constant. Every endeavour was made to keep within the lethality ranges of both drugs, or as close to them as possible. The number of the points again showed some fluctuation in accordance with the respective number of animals, depending as before upon the dispersion of the points.

Ethanol-hexobarbital. Ten points were determined, eight of them from a series of 40 animals and two of them from a series of 20 animals. The mortality percentage fluctuated between 30 and 95. Table 11.

TABLE 11

ETHANOL (x_1 ml/kg) — HEXOBARBITAL (x_2 mg/kg)

Dose x_1	Dose x_2	log x_1	log x_2	Number of animals	Number of deaths	Mortality percentage
5.8	210	0.763	2.322	40	12	30
	220		2.342	40	17	42.5
	230		2.362	40	21	52.5
	240		2.380	20	19	95
6.7	210	0.826	2.322	40	15	37.5
	220		2.342	40	21	52.5
	230		2.362	20	12	60
7.2	210	0.857	2.322	40	18	45
	220		2.342	40	27	67.5
	230		2.362	40	33	82.5

Ethanol-thiopental. Eleven points were determined, seven of them from a series of 40 animals. The mortality percentage fluctuated between 32.5 and 90. Table 12.

TABLE 12

ETHANOL (x_1 ml/kg) — THIOPENTAL (x_2 mg/kg)

Dose x_1	Dose x_2	log x_1	log x_2	Number of animals	Number of deaths	Mortality percentage
5.8	67	0.763	1.826	40	13	32.5
	71		1.851	40	16	40
	76		1.881	40	19	47.5
	80		1.903	20	16	80
6.7	67	0.826	1.826	40	20	50
	71		1.851	40	23	57.5
	76		1.881	20	12	60
7.2	67	0.857	1.826	40	24	60
	71		1.851	20	14	70
	76		1.881	40	34	85
7.6	67	0.881	1.826	20	18	90

Ethanol-morphine. Eleven points were determined, seven of them from a series of 40 animals, the rest from a series of 20 animals. The mortality percentage fluctuated between 20 and 82.5. Table 13.

TABLE 13

ETHANOL (x_1 ml/kg) — MORPHINE (x_2 mg/kg)

Dose x_1	Dose x_2	log x_1	log x_2	Number of animals	Number of deaths	Mortality percentage
5.8	110	0.763	2.041	40	8	20
	120		2.079	40	18	45
	170		2.230	40	26	65
6.7	110	0.826	2.041	40	18	45
	120		2.079	40	22	55
	170		2.230	20	12	60
7.2	150	0.857	2.176	20	14	70
7.6	110	0.881	2.041	40	22	55
	120		2.079	20	11	55
	170		2.230	40	33	82.5
8.0	110	0.903	2.041	20	13	65

Ethanol-atropine. Fifteen points were determined, six of them from a series of 40 and the rest from a series of 20 animals. The mortality percentage fluctuated between 15 and 85. Table 14.

TABLE 14

ETHANOL (x_1 ml/kg) — ATROPINE (x_2 mg/kg)

Dose x_1	Dose x_2	log x_1	log x_2	Number of animals	Number of deaths	Mortality percentage
5.8	130	0.763	2.114	20	3	15
	210		2.322	20	6	30
6.7	190	0.826	2.279	20	4	20
	240		2.380	20	14	70
	280		2.447	20	16	80
7.2	150	0.857	2.176	40	11	27.5
	190		2.279	40	20	50
	280		2.447	20	14	70
7.6	130	0.881	2.114	20	4	20
8.0	150	0.903	2.176	40	22	55
	190		1.279	20	12	60
	240		1.380	40	31	77.5
8.5	150	0.929	2.176	40	26	65
	190		2.279	20	14	70
	280		2.447	40	34	85

Ethanol-scopolamine. Thirteen points were determined, six of them from a series of 40 and the rest from a series of 20 animals. The mortality percentage fluctuated between 10 and 82.5. Table 15.

TABLE 15

ETHANOL (x_1 ml/kg) — SCOPOLAMINE (x_2 mg/kg)

Dose x_1	Dose x_2	log x_1	log x_2	Number of animals	Number of deaths	Mortality percentage
5.8	750	0.763	2.875	40	4	10
	780		2.892	20	5	25
	880		2.944	40	14	35
	980		2.991	20	10	50
6.7	750	0.826	2.875	20	4	20
	980		2.991	20	13	65
7.2	830	0.857	2.919	20	12	60
7.6	750	0.881	2.875	40	13	32.5
	780		2.892	20	8	40
	880		2.944	40	26	65
8.5	780	0.929	2.892	40	22	55
	880		2.944	20	14	70
	980		2.991	40	25	82.5

3. STATISTICAL TREATMENT OF THE RESULTS

The results of the experiments are presented in Tables 5—15. These results have been smoothed by determining the regression line or plane between the probit transformation of the mortality percentage and the logarithmic doses. Goodness of fit was tested with the chi square test (Finney 1952, Fisher and Yates 1957). The compatibility between the regression lines and the observation series is good, which means that the differences are not greater than would be expected considering random fluctuations. The results may be presented as follows:

$$\text{probit} = a + b \log_{10} x \text{ (one drug)}$$
$$= a_{12} + b_1 \log_{10} x_1 + b_2 \log_{10} x_2 \text{ (two drugs)}$$

where x, x_1 and x_2 are the doses expressed in the same units as in Tables 5—15. The values of the constants a and b, and a_{12}, b_1 and b_2 with their standard errors are shown in table 16, where the results of the compatibility tests are also presented. The connection between the probit scale and the percentage scale is given in table 17.

TABLE 16

Experiment	b	b_1 / b_2	a	a_{12}	x^2	f
Ethanol.....	11.1 ± 1.7	5.4 ± 1.7	— 5.9 ± 0.12	—54.8 ± 0.07	3.9	10
Hexobarbital .	14.1 ±	23.7 ± 4.0	—29.6 ±			
Ethanol.....	11.1 ± 1.7	9.1 ± 1.7	— 5.9 ± 0.12	—23.4 ± 0.07	7.7	7
Thiopental ..	9.3 ± 1.2	11.4 ± 3.0	—13.6 ± 0.08			
Ethanol.....	11.1 ± 1.7	5.3 ± 1.4	— 5.9 ± 0.12	— 7.7 ± 0.07	10.4	8
Morphine	3.2 ± 0.5	4.0 ± 0.9	— 2.8 ± 0.10			
Ethanol.....	11.1 ± 1.7	7.5 ± 1.5	— 5.9 ± 0.12	—10.3 ± 0.07	13.7	12
Atropine ..	4.1 ±	3.9 ± 0.6	— 5.6 ±			
Ethanol.....	11.1 ± 1.7	6.0 ± 1.1	— 5.9 ± 0.12	—28.5 ± 0.07	6.4	8
Scopolamine..	10.4 ±	9.7 ± 1.6	—26.6 ±			

4. ANALYSIS OF THE RESULTS

An analysis of the combined action of the drugs concerned in the light of the percentages of their lethal doses leads to the following main observation: If an ethanol dose of LD_{10} size is combined with

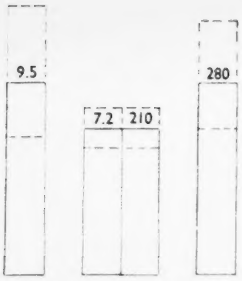
an equally strong dose (LD_{10}) of another drug, the resulting mortality rate is about 50 per cent for the combination ethanol-thiopental and even more than 75 per cent for the combination ethanol-hexobarbital. If the effects of the individual drugs were independent of each other, the hypothetical lethality of these combinations would be only 19 per cent. The difference between the observed lethality and the hypothetical one is of high statistical significance. On the other hand, however, it has to be borne in mind that if, for example, an LD_5 ethanol dose is combined with another LD_5 ethanol dose, the resulting lethality of this »combination» is about 100 per cent.

TABLE 17

%	0.1	0.5	1	5	10	15	20	30	40	50
	1.91	2.42	2.67	3.36	3.72	3.96	4.16	4.48	4.75	5.00
%	60	70	80	85	90	95	99	99.5	99.9	
	5.25	5.52	5.84	6.04	6.28	6.64	7.33	7.58	8.09	

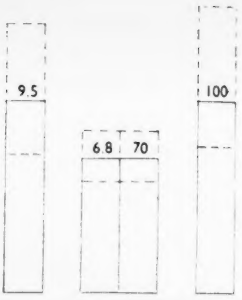
In Table 16 we see that irrespective of the drug the steepness coefficient of the lethality does not notably alter when the drug is given in combination with another one. Ethanol is an exception: its lethality curve is, on an average, significantly steeper when it is used alone than in the combinations. When we compare various combinations, again, there are no noticeable differences between the b_1 coefficients of ethanol. Even the rather high figure in the combination ethanol-thiopental may be due to random fluctuations. The use of ethanol in combinations with another drug thus renders it more effective on the one hand and on the other hand makes its lethality limits more vague. This implies that even rather small ethanol doses with low mortality rates seem to have a marked effect when combined with another drug. Thus an LD_2 amount of ethanol together with an LD_{10} amount of hexobarbital produces a mortality rate of 70 per cent. Hexobarbital seems to have a steeper lethality curve when used with ethanol than when given alone. The difference is not, however, statistically significant.

Diagrams (Figs. 12—16) have been drawn up to illustrate the results. In these diagrams the parallel columns denote the lethal doses of the individual drugs on the one hand and those of their combinations on the other (LD_5, LD_{50}, LD_{95}); in order to facilitate their comparison the combinations have been chosen in such a way



Ethanol ml. per kg. Hexobarbital mg. per kg.

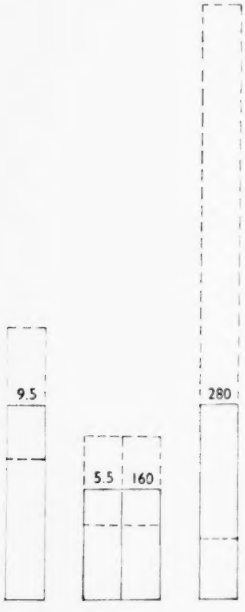
Fig. 12.



Ethanol ml. per kg. Thiopental mg. per kg.

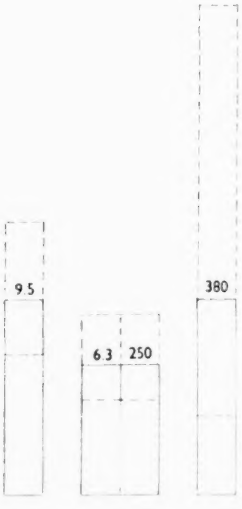
Fig. 13.

The highest column denotes 95 per cent lethality, the lowest column 5 per cent lethality, and the intermediate column (marked with a thick line) 50 per cent lethality.



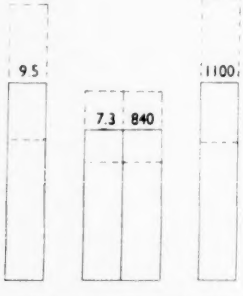
Ethanol ml. per kg. Morphine mg. per kg.

Fig. 14.



Ethanol ml. per kg. Atropine mg. per kg.

Fig. 15



Ethanol ml. per kg. Scopolamine mg. per kg.

Fig. 16

that each of the two constituents may be counted as having an equal share in the lethality. These columns also reveal the lethality steepness of the different drugs and their combinations, as also seen in Table 18, if the LD_5, LD_{50} and LD_{55} heights of each column are compared with each other. Table 18 shows the lethality steepness as a percentage. If the lethality percentage is small, like that of hexobarbital (30), it means that the range of the lethal doses of the drug concerned is less extensive than, for example, the range of morphine, the lethality steepness of which is 230 per cent. Morphine, in fact, has the widest lethality range of the drugs tested. This also affects the combined lethality steepness of ethanol and morphine by widening the lethality range and making their combined action more vague. The combined lethality steepness of ethanol and hexobarbital is 10 per cent, which means that the lethality range is very exact and that the quantitative difference between the LD_5 and the LD_{95} of the combination is small. The figures for the lethality steepness of the drug combinations used here are valid only in so far as the lethality rates of both drugs are about the same.

TABLE 18

THE STEEPNESS OF THE LETHALITY CURVES OF THE DRUGS AND OF THEIR COMBINATIONS

Ethanol	40 per cent		
Hexobarbital	30 » »	Ethanol-Hexobarbital	10 per cent
Thiopental	50 » »	Ethanol-Thiopental	20 » »
Morphine	230 » »	Ethanol-Morphine	50 » »
Atropine	150 » »	Ethanol-Atropine	40 » »
Scopolamine	40 » »	Ethanol-Scopolamine	30 » »

In this table the steepness of the effect means the percentage with which the 5 per cent lethal dose must be increased in order to obtain the 50 per cent lethal dose. Again, a proportionally equal increase in the 50 per cent lethal dose produces 95 per cent mortality. The lower the percentage the smaller the increase in the dose needed for higher lethality. This means that the lethal dose shows only slight variations between different laboratory animals.

In Figs. 17—21 the Gaddum method (1959) has been employed for comparing the same effects: the doses used are presented in a rectangular co-ordinative system. The type of the combined action of the drugs can be visualized from these figures through comparison of

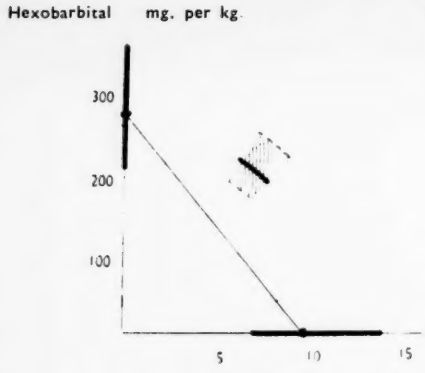


Fig. 17

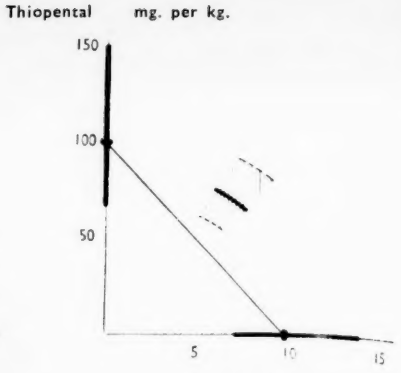


Fig. 18

The thickened segments of the axes indicate the 5—95 per cent lethality areas of the individual drugs. The hatched field denotes the corresponding lethality in the combined experiments. The blackened portion of the hatched field indicates the 50 per cent lethality.

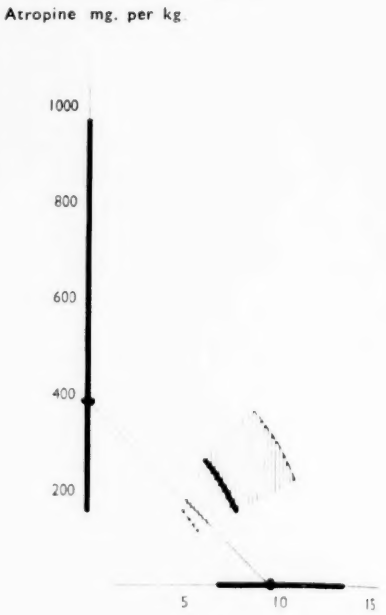


Fig. 20

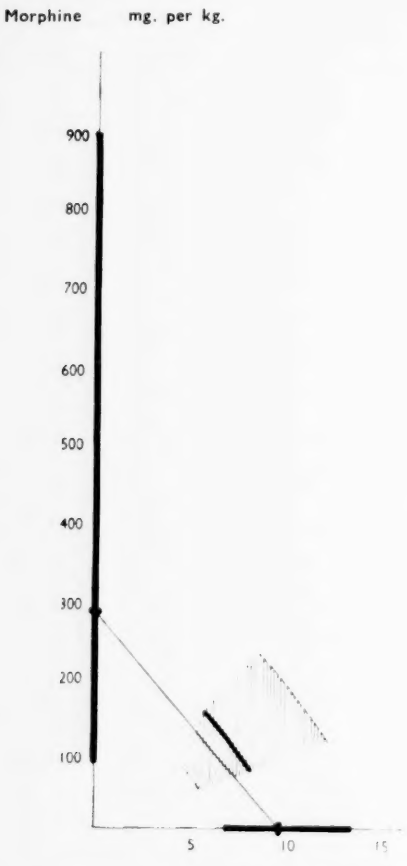


Fig. 19

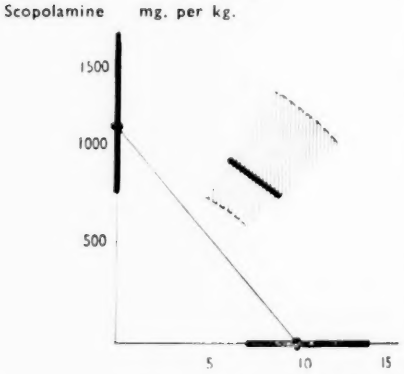


Fig. 21

the hatched field with the oblique line, which denotes the additive effect. The closer the lethality area is to the line, the stronger is the combined effect. The lethality areas of the individual drugs are represented by the thickened segments of the co-ordinate axes. The lethality steepness, seen in the columnar diagrams, can be observed here if the lengths of these segments are compared with each other.

A closer study of these two groups of diagrams, columns and co-ordinative systems reveals an additive synergism of all the combinations of the drugs concerned. The combined effect of the drugs investigated is slightly less than the algebraic sum of their individual effects. The combined effect of ethanol and morphine comes closest to the theoretically expected action. For no combination of the drugs is the combined column half of the height of the column denoting the individual effect of the respective drug. Nor does the hatched field fall inside the connecting line in any co-ordinate diagram. If it did the position would indicate the existence of a potentiative kind of synergism.

V. DISCUSSION

The combined action of two drugs is dependent on a great many factors. This renders it very difficult to estimate the combined effect. It is also easy to understand why there is such a great variety and diversity of terminology in the literature. Even when using or testing one drug alone, we have to distinguish between its therapeutic, pharmacological and toxic actions. This distinction becomes even more important when the combined action of two drugs is concerned. It is often difficult to draw the line between the pharmacological and the toxic effects, because it mostly depends on the dosage or the method of administration. In these circumstances the margin may be very slender indeed, or alternatively great.

In the investigations which deal with the toxicity of the combined action of ethanol and barbiturates the sleeping time or death of the laboratory animals is used as the criterion of toxicity. Quite a large number of different methods have been adopted for determining the sleeping time. Arvola *et al.* (1958) stated that quite a number of demands must be fulfilled before an ideal method was found. Gruber (1955) also criticized the use of the sleeping time as the criterion for the combined action of ethanol and barbiturates. Though both ethanol and barbiturates produce depression of the central nervous system, which can be measured by the duration of the sleeping time, there is nevertheless no reliable means of comparing these sleeping times. Though a certain dose of one drug gives a double effect in comparison with another drug, the duration of the former effect need not necessarily be double the duration of the latter. Each of these two drugs has its own efficiency threshold, which has to be crossed before the duration of the effect can be measured. Since it is exceedingly difficult to determine the position of this threshold it is equally difficult to ascertain the dose of a

drug which will produce a measurable effect. A cautious and critical attitude must thus be taken in regard to the results of the investigations in which the sleeping time was used as the criterion.

In their investigations of the combined action of ethanol and pentobarbital Carrière *et al.* (1934) came to the conclusion that these drugs were antagonistic. Their experiments on animals did not, however, give convincing support to the results reported, especially as the duration of the barbiturate coma without the effect of ethanol was not mentioned in the paper. Furthermore, it has to be considered that Allegri's (1935) re-investigation did not confirm the results attained by Carrière *et al.* (1934). Olszycka (1935, 1936), again, reported, on the basis of her animal experiments, the existence of potentiation between ethanol and barbiturates. There are, however, considerable inconsistencies in her experiments. When, for example, her laboratory animals were given ethanol in combination with increasing amounts of butethal, she computed at 56.0 the coefficient for an ethanol dose of 3.2 mg. per gm. plus 0.06 mg. of butethal per gm.; while for an ethanol dose of 3.2 mg. per gm. in combination with 0.03 mg. of butethal per gm. the value of the coefficient was 14.5. The only conclusion to be drawn from this investigation is the existence of synergism between the drugs concerned; but it is impossible to infer from the results reported what kind of synergism is in question.

According to several investigations on laboratory animals the combined action of ethanol and barbiturates would be of the potentiative type (Dille and Ahlquist 1937, Jetter and McLean 1943, Seeberg and Dille 1943, Ramsey and Haag 1946, Sandberg 1951, Smith and Loomis 1951). This view is supported by clinical observations on man. The potentiative effect of ethanol and barbiturates was regarded as the cause of death in several cases reported by Jetter ans McLean (1943), Fisher *et al.* (1948), Burrows (1953).

Earlier experimental studies were criticized by Gruber (1955). In his terminology synergism is synonymous with potentiation. On the basis of his experiments on animals the combined action of ethanol and barbiturates was a simple addition. Gruber's opinion was supported by later investigators, though they used differing terms (Fearn and Hodges 1953, Archer 1956, Aston and Cullumbine 1959).

On the ground of their clinical experience Moller (1952) and

Naalsund (1955) considered the combined effect of ethanol and morphine to be of the potentiative type. The same result was obtained with Polamidon by Wagner and Wagner (1958). In their experimental studies on mice Eerola *et al.* (1955) and Venho *et al.* (1955) also regarded the combined action of morphine and ethanol as distinctly potentiative.

In the present investigation analyzing the effect of ethanol in combination with hexobarbital, thiopental, morphine, atropine and scopolamine, I have aimed at the greatest possible simplicity and reliability in the arrangement of the experiments. The mouse was the only possible animal for these experiments, because large amounts of animals were needed. Its sensitivity in alcohol experiments does not differ from other laboratory animals. Although its great resistance to atropine and scopolamine was known to the author, the mouse was nevertheless selected, because only sublethal or lethal doses were used.

Death has been used as the criterion of toxicity, because its verification is much more reliable than, for example, that of the sleeping time.

According to Robinson (1945), an observation period of 20 minutes should be long enough to verify the cases of death after the intravenous administration of thiopental. Ramsey and Haag gave their test animals a control period of 48 hours. In the present series the control period was 24 hours, which was considered to be the most suitable as regards the method of administration and the results of the preliminary experiments.

Aston and Cullumbine (1959) discovered that with the combined intraperitoneal administration of ethanol and secobarbital the greatest toxicity was reached when the drugs were given immediately after each other. After preliminary experiments the author came to confirmatory conclusions. In the combined experiments ethanol was given first, because the action of swallowing performed by a conscious mouse facilitates the administration.

The reaction of the mouse in the experiments with one drug was generally similar to the reaction in the combined experiments. The mouse peacefully lost consciousness, and, with an increasing central depression, its breathing gradually ceased and the animal died. An exception was morphine with its typical symptoms of intoxication, which, however, were absent or subdued when mor-

phine was given in combination with ethanol. Another exception was thiopental, after the administration of which the mouse was usually restless and had several attacks of convulsions. Thiopental was an exception to the other drugs in another respect, too: in a few cases the mouse was able to recover as late as after 48 hours' unconsciousness. When thiopental was administered in combination with ethanol these symptoms grew weaker or disappeared altogether. The experimental results give evidence that the mice died from breathing difficulties due to central depression in all the experiments with one or two drugs.

The combined effects of all the sublethal and lethal doses administered in the present experiments were synergistic. In all the experiments with ethanol in combination with the other drugs concerned this synergism appeared to be of the additive type. This is clearly and consistently proved by the columnar diagrams and the co-ordinate systems of the Gaddum type. On the other hand, everything stated above serves to support the view that the effects on the organism of the drugs tested in toxic doses are very much alike.

Though the results of these experiments on mice are in no way directly applicable to man, they nevertheless prove that ethanol is a substance with a strong depressive effect on the central nervous system; and that in combination with another drug of similar effect it may produce death even though given in doses which would not be lethal when administered separately. This has also been proved by clinical observations (Jetter and McLean 1943, Fisher *et al.* 1948, Burrows 1953, Møller 1952, Naalsund 1955), whereas it has not been proved whether the synergism between ethanol and the drugs examined here is of the additive or of the potentiative type in man. It has to be emphasized, however, that even if it is of the additive kind, the share of ethanol must not be forgotten when hexobarbital, thiopental, morphine, atropine, or scopolamine is being administered to a person under the influence of ethanol.

The following generalization can be made: When two drugs are administered simultaneously to man their combined action must always be considered. This may be synergism or antagonism; or the drugs may act independently, in which case their actions may also be summed up as untoward effects — effects which were not expected.

VI. CONCLUSIONS

Experiments on mice have clearly shown that the combined effect of sublethal or lethal doses of the drugs investigated

1. ethanol and hexobarbital
2. ethanol and thiopental
3. ethanol and morphine
4. ethanol and atropine
5. ethanol and scopolamine

is synergistic, and that the synergism is of the additive type.

VII. SUMMARY

There is a certain lack of uniformity in pharmacological nomenclature when the combined action of two drugs is referred to. In the introductory part of his book the present writer discusses briefly the terms used in some medical handbooks and dictionaries.

The literature contains reports of cases of sudden and unexpected death under the influence of ethanol and barbiturates. A number of cases of sudden death have also been reported as most probably due to the combined effect of ethanol and morphine.

Through experiments on mice the author of the present investigation attempts to throw light on the following problems: Is the combined effect of ethanol and hexobarbital, thiopental, morphine, atropine, or scopolamine antagonistic or synergistic? If synergistic, is the synergism of the additive or of the potentiative type?

The authors of the experimental studies published before have not agreed about whether there is an antagonism, an additive synergism or a potentiative synergism between ethanol and barbiturates when given in combination. An account is given of the investigations reported in the literature.

Two experimental studies on animals demonstrate the existence of potentiation between ethanol and morphine. No experiments have been made with atropine or scopolamine in combination with ethanol.

The present investigation is based on experiments on some 2,900 mice. Ethanol was given orally according to the Behrens method; immediately after the ethanol the other drug was injected subcutaneously under the skin of the neck.

The LD_5—LD_{95} were first determined for each drug separately; then the LD_5—LD_{95} were determined for the combinations of ethanol with each of the other drugs.

The results were submitted to mathematical treatment and represented in columnar diagrams and in Gaddum's co-ordinate system. The final results of the investigation revealed the existence of additive synergism between all the combinations of the drugs examined, when given in sublethal or lethal doses. The combined effect of the drugs concerned was slightly less than the algebraic sum of their individual effects. The combined effect of ethanol and morphine came closest to the algebraic sum. For no combinations of the drugs was the combined column half the height of the column denoting the individual effects of the respective drugs; nor did the area of the combined action fall inside the connecting line in any co-ordinate diagram. The latter would have indicated the existence of potentiative synergism.

The results of a series of experiments on mice are in no way directly applicable to man.

It remains to be proved whether the synergism between ethanol and the drugs examined is of the additive or of the potentiative kind in man. It has to be pointed out, however, that even if the synergism is of the additive type, the additive share of ethanol must not be forgotten when a person under the influence of ethanol is being given hexobarbital, thiopental, morphine, atropine or scopolamine.

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